ABSTRACT

SCHWARZ, TANNER. Distribution, Virulence, and Sweetpotato Resistance to *Meloidogyne enterolobii* in North Carolina. (Under the direction of Dr. Eric Davis).

Meloidogyne enterolobii is an aggressive root-knot nematode (RKN) species that has been detected in North Carolina (NC) within the last decade. Its ability to infect a wide range of host plants and overcome root-knot nematode resistance genes has threatened economic crop production. In collaboration with the North Carolina Department of Agriculture (NCDA) Nematode Assay Lab, DNA from RKN samples from economic crops grown in the eastern-half of North Carolina were analyzed by polymerase chain reaction (PCR) to assay for the presence of *M. enterolobii*. This species was detected in Columbus, Sampson, Harnett, Johnston, Wayne, Greene, Wilson, and Nash counties in this survey. Three populations of *M. enterolobii* from NC sweetpotato farms, and one population from a NC soybean farm have been cultured on roots of greenhouse tomato plants (*Lycopersicon lycopersicum* L.). A study to assess potential differences in virulence among the four cultured North Carolina populations of *M. enterolobii* on six selected sweetpotato [*Ipomoea batatas* (L.) Lam.] genotypes found no difference in virulence among the four populations of *M. enterolobii* that were tested. Potential resistance to *M. enterolobii* in 91 selected sweetpotato genotypes was evaluated in greenhouse experiments. Genotype susceptibility to *M. enterolobii* was assessed as the number of nematode eggs per gram of root. Sweetpotato genotype was significantly different ($P < 0.001$) for gall rating, total eggs, and eggs per gram of root based on mean separations, and based upon Fisher's LSD *t* test (alpha=.05), sweetpotato genotypes that supported 500 eggs/gram root or less were classified as resistant to *M. enterolobii.* Twenty out of the 91 sweetpotato genotypes tested were considered resistant based on 500 eggs/gram root or less of nematode reproduction, with most resistant genotypes supporting less than 20 eggs/gram root of *M. enterolobii*. Susceptible genotypes

included ‘Covington’ averaging 3,730 eggs per gram of root, ‘Beauregard’ at 4,263, ‘NCDM04-001’ at 19,613, and ‘HiDry’ at 9,925 eggs/gram root. Resistant sweetpotato genotypes included ‘Tanzania’ at 1.81 eggs/gram root, ‘Murasaki-29’ at 2.98, ‘Bwanjule’ at 5.06, ‘Dimbuka-Bukulula’ at 11.30, ‘Jewel’ at 1.91, ‘Centennial’ at 2.87, and ‘Tib-11’ with 10.77 eggs/gram root. The origins and genetic background of the different resistant sweetpotato genotypes suggested that multiple sources of resistance to *M. enterolobii* may exist within the sweetpotato germplasm examined. A collaboration with NC State sweetpotato breeders has been established to incorporate the observed resistance to *M. enterolobii* into commercial sweetpotato cultivars.

Distribution, Virulence, and Sweetpotato Resistance to *Meloidogyne enterolobii* in North Carolina

by
Tanner Schwarz

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APPROVED BY:

_____	_____
Eric Davis Committee Chair	Weimin Ye
_____	_____
Lina Quesada-Ocampo	Lindsey Thiessen

DEDICATION

I would like to dedicate this document to my parents, Sherry Schwarz and Dr. Michael Schwarz, my brother Ryan Schwarz, and my sister Megan Schwarz. Thank you for your support and belief through my life.

BIOGRAPHY

Tanner Schwarz was born on December 21, 1993 in Overland Park, Kansas. He completed his Bachelor of Science in Biology from the University of North Carolina Wilmington in 2016. In the fall of 2016, Tanner accepted a graduate position at the North Carolina State University in the Department of Entomology and Plant Pathology to pursue his Master of Science.

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Chapter 2. Distribution of *Meloidogyne enterolobii* in Eastern North Carolina and Comparison of Four Isolates

Literature Review

Introduction to Nematodes

Nematodes are unsegmented roundworms belonging to the phylum Nematoda (Kionte and Fitch, 2013). In Greek, ‘nema’ means thread, in which threadlike is often used to describe the general nematode body (Kiontke and Fitch, 2013). Nematodes are vermiform, or worm-shaped, in all or part of their life cycle, and some species can become rounded and swollen in older life stages (Lambert and Bekal, 2002). About 30,000 species have been described, with an estimated number of total species to be over a million (Kiontke and Fitch, 2013). Nematodes are the most abundant animal in the world, as four out of every five animals are nematodes (Platt and Lorenzen, 1994). With such abundance, nematodes are very diverse in terms of their size, lifestyle, habitat, and diet (Agrios, 2005). Nematodes must live in an aqueous environment and can range from sizes of just fractions of a millimeter to several feet long (Carter and Sasser, 1985; Lambert and Bekal, 2002; Agrios, 2005). Most nematode species are considered free-living microbivores, which live freely in soil moisture or bodies of water, but some species have a parasitic lifestyle in which animals or plants can be the host (Agrios, 2005). About 40% of described species are considered free-living, about 44% parasitize animals, and 15% parasitize plants (Lambert and Bekal, 2002). Despite the vast and diverse number of nematode species, all embryonate in an egg and then go through four juvenile stages (Sasser and Carter, 1985). At the end of each juvenile stage, the nematode forms a new cuticle and molts the old one (Sasser and Carter, 1985). After the fourth molt, a nematode reaches the final, reproductive adult life stage (Kiontke and Fitch, 2013). Success of nematodes can be attributed to characteristics that have likely been evolved and articulated over time. These characteristics include a protective cuticle,

facultative diapause, biochemical adaptations, and various reproductive strategies that can contribute to the nematode's ability to adapt to extreme conditions (Blaxter and Bird, 1997).

Nematodes have adapted unique characteristics to deal with environmental factors and enhance survival. One important characteristic is the cuticle (Cater and Sasser, 1985; Blaxter and Bird, 1997). Other organisms contain a cuticle, but nematodes have no internal skeletal structure. The cuticle acts against internal turgor pressure to maintain body shape and to aid in locomotion (Lambert and Bekal, 2002). Muscles are attached longitudinally to the nematode's hypodermis, allowing it to move (Lambert and Bekal, 2002). In certain times of environmental stress, such as a lack of nutrients or overcrowding, nematodes can enter a dauer or diapause stage (Carter and Sasser, 1985; Chitwood, 2003). This is a quiescent form that can occur in an early juvenile stage, typically in stressful situations, that aids in long-term survival (Chitwood, 2003). The dauer stage is a developmentally arrested inactive state in which these nematodes have impermeable cuticles and enclosed openings that allow them to survive for months until extrinsic and intrinsic conditions are favorable (Blaxter, 2011).

Plant-Parasitic Nematodes

Plant-parasitic nematodes are obligate biotrophs, meaning a host plant is necessary for the nematode to grow and reproduce to complete its life cycle (Williamson and Hussey, 1996). They parasitize and feed off the nutrients of the cytoplasm of plant cells (Williamson and Hussey, 1996). Not all nematodes are adapted to parasitize and infect plants. Plant-parasitic nematodes have an oral stylet, which is a protrusible hollow needle-like structure (Perry, 1996). It is used to probe plant tissues and ingest nutrients from plant cells, and also serves as the pathway to secrete proteins and other small molecules, called effectors, that are essential in the

infection process (Mitchum et al., 2013). Effectors have been defined by Hogenhout et al. (2009) as ‘all pathogen proteins and small molecules that alter host cell structure and function’. However, despite knowing the importance, the identification and function of many nematode effectors are still largely unknown (Mitchum et al., 2013).

Most plant-parasitic nematodes are soilborne pathogens that target and infect living plant roots in large numbers, which often causes severe root damage that can lead to above-ground symptoms such as nutrient deficiency, stunting, wilting, reduced yield and sometimes plant death (Anonymous, 2014; Ye, 2018). In order to locate a host plant, plant-parasitic nematodes follow chemotactic signals (Reynolds et al., 2011). Chemotaxis is a movement towards higher concentrations of chemicals, such as plant root exudates (Reynolds et al., 2011). These chemical signals are soluble or gaseous attractants which are produced by the roots that nematodes have adapted to follow (Reynolds et al., 2011). A study conducted by Reynolds et al. (2011) showed that nematodes took the shortest route to their preferred hosts through chemotaxis signals; however, nematodes took the longest route towards poor hosts. These results provide evidence that nematodes are able to locate a preferred host and move directly towards the signals, highlighting adaptations to parasitize specific host plants (Reynolds et al., 2011).

Plant-parasitic nematodes can be grouped into either ectoparasites or endoparasites (Lambert and Bekal, 2002; Agrios, 2005). Ectoparasites remain outside of the host and use their stylet to feed from plant cells (Lambert and Bekal, 2002). This feeding strategy allows a nematode to potentially target numerous parts of a root system or neighboring plants, allowing it to switch hosts more easily (Sasser and Carter, 1985). Ectoparasitism can present dangers for the nematode because it is more exposed to predators and less protected from environmental changes (Lambert and Bekal, 2002). Endoparasites are able to penetrate and feed within plant root tissue

(Lambert and Bekal, 2002). Endoparasites can be either migratory, in which they can move throughout the root tissue feeding on plant cells, or they can be sedentary, in which the nematode establishes a more permanent feeding site (Chitwood, 2003). Ectoparasites and endoparasites are both destructive pathogens; however, sedentary endoparasites are considered the most economically damaging nematodes on a global scale (Perry, 1996; Agrios, 2005). Ectoparasites include ring (*Criconemella* spp.), dagger (*Xiphinema* spp.), stubby root (*Trichodorus* spp.), and sting (*Belonolaimus* spp.) nematodes. Migratory endoparasitic nematodes include lesion (*Pratylenchus* spp.), stunt (*Tylenchorhynchus* spp.), lance (*Hoploaimus* spp.), and spiral (*Helicotylenchus* spp.) nematodes (Sasser and Carter, 1985; Agrios, 2005). The most prominent nematodes that have a sedentary endoparasitic lifestyle are cyst (*Heterodera* and *Globodera*) and root-knot (*Meloidogyne*) nematodes (Agrios, 2005).

Among the unique characteristics that make plant-parasitic nematodes successful are the various reproductive strategies, depending on the species, which include mitotic parthenogenesis, meiotic parthenogenesis, and amphimixis (Sasser and Carter, 1985; Chitwood and Perry, 2009). In mitotic parthenogenesis, diploid chromosomes replicate, and the nucleus separate into the two daughter nuclei, with each having the full complement of chromosomes (Chitwood and Perry, 2009). It involves no reduction division (Sasser and Carter, 1985). This is a clone of the original genetic material unless mutations occur. In meiotic parthenogenesis, chromatids of each diploid pair separate, and each pair remains in the nucleus and the other near the cell membrane (Chitwood and Perry, 2009). This involves a reduction division (Sasser and Carter, 1985). This results in a haploid that can be fertilized by a male where genetic recombination can occur. If not fertilized, the result should be clones of the female (Chitwood and Perry, 2009). The third and most common nematode reproductive strategy is amphimixis (Sasser and Carter, 1985; Chitwood

and Perry, 2009). Amphimixis is sexual reproduction in which the haploid spermatocyte fertilizes the haploid oocyte, which results in genetic recombination (Sasser and Carter, 1985; Chitwood and Perry, 2009). These various reproductive strategies allow for reproduction to occur with or without the presence of a male, which contribute to the success of nematode survival (Blaxter and Bird, 1997; Chitwood and Perry, 2009).

Impact of Plant-Parasitic Nematodes

There are over 4,100 species of described plant-parasitic nematodes (Jones et al., 2013). They are found all around the world and can cause serious damage and add constraints to the global plant and agricultural industry (Sasser and Carter, 1985). It is estimated that over $100 billion is lost annually and approximately 8-15% of crop loss worldwide is due to nematodes (Jones et al., 2013). These figures are most likely an underestimation because growers, especially in under-developed countries, may be unaware of the presence of plant-parasitic nematodes infecting their crops, making it difficult to assess the potential yield versus the actual yield of an infected crop (Agrios, 2005). Plant-parasitic nematodes are among the greatest threat to crops globally, as all crops are susceptible to damage by at least one nematode species (Ye, 2018). Different species of plant-parasitic nematodes have been found to infect almost every part of a host plant, including roots, shoots, bulbs, leaves, fruits, and seeds (Sasser and Carter, 1985; Ye, 2018), although the majority are root-infecting species. With a wide host range and potential to cause high damage, plant-parasitic nematodes present a global threat to crop production (Sasser and Carter, 1985).

In addition, nematodes have the potential to form disease complexes that increase the severity of infection and damage to the host plant (Sasser and Carter, 1985; Agrios, 2005). Since

plant-parasitic nematodes live in soil, they are constantly surrounded by bacteria and fungi that can develop an association (Agrios, 2005). One potential mechanism is wounding of plant roots can occur when nematodes enter the roots to feed (Agrios, 2005). Wounding from nematode attack is often an opportunistic opening for other pathogens, such as *Rhizoctonia solani*, to infect the host plant and cause an increase in root damage and above ground symptoms (Al-Hazmi and Al-Nadary, 2015). A study was conducted to assess the interaction between *Meloidogyne incognita* and *Rhizoctonia solani* and their infections on green beans (*Phaseolus vulgaris*) (Al-Hazmi and Al-Nadary, 2015). They found that if inoculating green beans with both *M. incognita* and *R. solani* at the same time, both root rot caused by the fungus, and the root galls caused by the nematode, were increased in comparison to single species inoculation without the interaction. However, root rot and root gall severity were much greater when *M. incognita* was inoculated first, and two weeks later followed by inoculation with *R. solani* (Al-Hazmi and Al-Nadary, 2015). Nematodes can also form an interrelationship with viruses (Agrios, 2005). Viruses can be transmitted by nematodes, such as the tomato ringspot virus can be transmitted by *Xiphinema* spp. (Dagger nematode). Nematodes can transmit a virus after feeding on virus-infected plants as results suggest transmission can occur after only one hour of feeding (Agrios, 2005).

Root-knot Nematodes

One of the most widespread pathogens reducing agricultural productivity throughout the world are the root-knot nematodes, *Meloidogyne* species (Sasser and Carter, 1985). Almost all the plants that the world relies upon for food supply are susceptible to infection by one or more RKN species (Sasser and Carter, 1985; Ye, 2018). There are about 100 described species of *Meloidogyne* that can infect a very wide range of host species (Mitkowski and Abawi, 2003). It

can sometimes be challenging to diagnose crops infected by RKNs (Sasser, 1952; Sasser and Carter, 1985); often, growers never realize they have a nematode problem until the end of the season when growers harvest crops and notice damaged commodities and galled roots (Sasser, 1952). It can be difficult to incorporate a rotational crop plan since many plant species are susceptible to RKN species (Sasser and Carter, 1985; Agrios, 2005; Lambert and Bekal, 2002). In addition, nematodes can infect alternative hosts such as many weed species to survive, in some instances, for many years (Sasser and Carter, 1985; Agrios, 2005). RKN are common pathogens that represent a global threat to crop production (Sasser and Carter, 1985; Karuri et al., 2017; Ye, 2018).

Life Cycle of *Meloidogyne* spp.

The first molt occurs in the egg and second stage juveniles (J2) hatch from the egg into the soil (Figure 1) (Sasser and Carter, 1985; Chitwood, 2003). Second stage juveniles are the only infective stage, meaning only J2s can migrate and initiate an infection in a host plant (Sasser and Carter, 1985; Chitwood and Perry, 2009). These juveniles are attracted to roots by chemotaxis signals from root exudates (Chitwood, 2003; Reynolds et al., 2011). The juvenile will follow these signals until it reaches the zone of elongation in the roots, where it penetrates and travels intercellularly to the vascular cylinder (Reynolds et al., 2011). Once the nematode identifies suitable plant cells for a feeding site, it releases effectors through its stylet into selected plant cells (Mitchum et al., 2013). In response, plant procambial cells develop into ‘giant cells’, which are enlarged multinucleate cells that serve as the permanent feeding site for the pathogen (Sasser and Carter, 1985). Each nematode can establish five to seven giant cells to form around the nematode head, each containing up to 100 enlarged nuclei (Williamson and Hussey, 1996).

During this process, swelling and division of cortical cells around the feeding site leads to formation of intercalary root galls (Williamson and Hussey, 1996). Galls are characteristic symptoms of *Meloidogyne* infection that are a response by the host plant to the nematode (Figures 2 and 3) (Sasser and Carter, 1985; Chitwood, 2003).

The nematode swells and becomes sedentary once it establishes a feeding site within the root and feeds from the plant cells (Williamson and Hussey, 1996; Chitwood, 2003). *Meloidogyne* J2s progress through a series of molts to become pear-shaped adult females (Williamson and Hussey, 1996). The nematode rapidly molts through the J3 and J4 stage in which feeding is paused. Under certain conditions, such as a lack of available nutrition or over-crowding, the nematode can develop into an adult vermiform shaped male, which exits the root without feeding (Chitwood, 2003). However, adult male formation is rare, and in most cases, J4s develop into swollen round-shaped females (Chitwood, 2003; Agrios, 2005). These females mature and lay their eggs in a gelatinous matrix on the outside surface of the root which helps protect the eggs (Sasser and Carter, 1985; Williamson and Hussey, 1996). A single female can lay numerous eggs ranging from 500 to over 1,000 (Lambert and Bekal, 2002). *Meloidogyne* eggs usually hatch at random and do not require the presence of root exudates, although cold conditions can slow nematode development (Williamson and Hussey, 1996). A single *Meloidogyne* life cycle takes about 25 days to complete under favorable conditions, and thus, several generations can occur during a single growing season (Lambert and Bekal, 2002).

International *Meloidogyne* Project

The International *Meloidogyne* Project (IMP) was formed in 1975 in order to assist in developing countries to increase yield of economic crops by research into biology and

management of RKN (Sasser and Carter, 1985). The IMP had received more than 1800 *Meloidogyne* populations representing 16 species (Sasser et al., 1983). The IMP produced many important findings in Nematology, particularly RKN. They identified methods that can be used to identify species, such as the North Carolina differential host test, biochemistry, and morphology techniques (Sasser and Carter, 1985). The North Carolina differential host test distinguishes populations of *Meloidogyne* based on their reactions on host plants. For example, the IMP found that *M. arenaria* has two races, in which race 1 comprises 16% of all race 1 isolates. Race 1 reproduces on peanut; however, race 2 comprises 84% of *M. arenaria* populations and does not reproduce on peanut (Sasser et al., 1983; Dong et al, 2007). The IMP also concluded that 95% of all infestations on plant crop hosts in cultivated land is due to infection from four major *Meloidogyne* species; *M. incognita, M. javanica, M. arenaria,* and *M. hapla* (Sasser et al., 1983; Sasser and Carter, 1985). Some of the more significant research in the IMP assessed different options to control and manage RKN. Many methods of control were investigated, but the IMP concluded that cropping systems research into non-hosts and development of resistant cultivars are the most environmentally friendly and economically efficient methods that provide control of RKN (Sasser and Carter, 1985). Overall, the IMP was able to increase awareness to the threat of RKN globally, especially in developing countries. The discovery of new species, races, identification techniques, and management practices by the IMP has formed a basis for RKN knowledge to conduct and improve research to properly manage RKN (Sasser et al., 1983; Sasser and Carter, 1985).

Plant-Parasitic Nematode Management

Once plant-parasitic nematodes establish themselves in a field, it is very difficult to eliminate the populations to non-threatening economic thresholds or eradicate them (Sasser and Carter, 1985). Therefore, it is vital for a grower to prevent the introduction of nematodes to their field. The majority of plant-parasitic nematode species are only able to move no more than a meter in the soil (Lambert and Bekal, 2002; Agrios, 2005). Most cases of nematode movement from field to field or over long distances occur through human involvement (Agrios, 2005; Lambert and Bekal, 2002). Farm equipment, such as tractors, or muddy boots can harbor nematode infested soil that can easily be transferred to previously nematode-free crops and fields (Agrios, 2005). If proper precautions are not met, such as sanitizing tractor wheels or dirty boots, nematodes can be introduced into a field and reproduce to thresholds that cause economic crop loss (Sasser and Carter, 1985; Lambert and Bekal, 2002).

The chance of transporting nematode-infected plant material increases with global plant trade (Anonymous, 2008). Growers often buy plant material, such as vegetable transplants, to be planted during the current growing season. These plant materials or the soil media can sometimes harbor nematodes (Agrios, 2005; Anonymous, 2008; EPPO, 2009). Certified disease-free plant material programs have been formed to reduce the instances of nematodes or other pathogens from being distributed, such as the European and Mediterranean Plant Protection Organization (EPPO) (Anonymous, 2008; EPPO, 2009). Although purchase of these materials through programs can sometimes be expensive, the risk of economic damage by nematode infestation becomes more expensive once the nematode is introduced into the field (Agrios, 2005; Anonymous, 2008; EPPO, 2009).

In fields with known nematode infestations, management strategies are intended to reduce nematode population levels at the beginning of a growing season or to reduce reproduction rate during the growing season to help control nematodes below their threshold of economic damage (Chitwood, 2003; Agrios, 2005; Ntalli and Caboni, 2012; Ebone et al., 2019). The use of nematicides provides one option to reduce initial populations of nematodes in a field (Chitwood, 2003; Agrios, 2005; Ntalli and Caboni, 2012; Baidoo et al., 2017). The purpose of chemical nematicides is to reduce nematode population densities below economic damage thresholds (Sasser and Carter, 1985; Chitwood, 2003). Chemical control of nematodes has been used since the late 19th century (Chitwood, 2003; Ntalli and Caboni, 2012; Ebone et al., 2019). The term nematicide is used most often but encompasses as a chemical used to control nematodes. If a chemical is lethal to nematodes, then it is a nematicide, in technical terms (Chitwood, 2003; Ntalli and Caboni, 2012; Ebone et al., 2019). If a chemical adversely alters nematode physiology and behavior but can be reversed upon removal of the chemical, it is considered a nematistat (Chitwood, 2003; Ebone et al., 2019). Nematicide will be the term used in this treatise to include both nematistats and nematicides. Nematicides can be categorized as either fumigants or non-fumigants (Chitwood, 2003; Ebone et al., 2019).

Fumigant nematicides are injected into soils and move through the soil as a volatile gas (Chitwood, 2003; Agrios, 2005; Ebone et al., 2019). Fumigants have a low binding affinity, so they typically do not have a long residual activity (Chitwood, 2003). Fumigants came about in the second half of the 19th century with the use of carbon disulfide (Chitwood, 2003; Vang et al., 2016; Ebone et al., 2019). However, use became more popular following World War I due to the surplus of nerve gas (Chloropicrin), which showed efficacy on nematodes and other soil pathogens. D-D (a mixture of 1,3-dichloropropene and 1,2-dichloropropane) controlled soil

pathogens and led to increases in crop yield, which ignited research into fumigants to reduce agricultural pests and pathogens (Chitwood, 2003; Ebone et al., 2019). Soon, a series of other halogenated hydrocarbons and other volatile compounds were developed as soil fumigants (Chitwood, 2003; Ntalli and Caboni, 2012; Baidoo, 2017; Ebone et al., 2019).

Some fumigants such as methyl bromide and chloropicrin are general biocides, meaning they kill a broad range of pests, pathogens, weeds, and non-target organisms (Chitwood, 2003; Baidoo et al., 2017; Ebone et al., 2019). Since many soil fumigants are phytotoxic, they are commonly applied as pre-plant treatments (Chitwood, 2003; Ntalli and Caboni, 2012; Faske and Hurd, 2015). Some fumigants have more specific activity against nematodes and soil invertebrates such as 1,3-dichloropropene (Telone II) but are still chiseled into the soil pre-plant due to phytotoxicity (Chitwood, 2003; Ntalli and Caboni, 2012; Faske and Hurd, 2015). Fumigants can be very effective in reducing large nematode populations, however, their risk to the environment has resulted in restrictions in the type and number of fumigant nematicides currently available (Agrios, 2005; Chitwood, 2003; Ntalli and Caboni, 2012; Vang et al., 2016; Baidoo et al., 2017; Ebone et al., 2019).

Non-fumigant nematicides are formulated as liquids or granules that move through the soil with water rather than high vapor pressure (Chitwood, 2003; Ntalli and Caboni, 2012). They typically have a narrower spectrum of activity than fumigants (Faske and Hurd, 2015). In the 1960's, non-fumigant carbamates and organophosphates were introduced as contact nematicides because of their activity in animal nervous systems (Ebone et al., 2019). These chemicals bind with acetylcholinesterase (AChE) which results in accumulation of acetylcholine at nerve synapses, which leads to paralysis of the nematodes (Chitwood, 2003; Baidoo et al, 2017; Ebone et al., 2019). The nematodes are unable to locate a host and eventually die (Chitwood, 2003;

Agrios, 2005; Ntalli and Caboni, 2012; Baidoo et al., 2017). Non-fumigants can also be extremely toxic to humans, mammals, and the environment especially if not properly used, however, are typically less toxic than fumigants since the fumigant is a volatile gas that is more difficult to control (Chitwood, 2003; Faske and Hurd, 2015; Ebone et al., 2019). However, non-fumigants tend to be easier to apply, can be applied post-plant, and some have systemic activity within the plant (Chitwood, 2003; Agrios, 2005; Faske and Hurd, 2015). Non-fumigants include products like abamectin, oxamyl, aldicarb, fluensulfone, fosthiazate, and fluopyram (Chitwood, 2003; Faske and Hurd, 2015; Morris et al., 2016; Vang et al., 2016; Ebone et al., 2019).

Chemical control is a great resource to manage nematodes; however, complete eradication is unlikely (Sasser and Carter, 1985; Chitwood, 2003; Agrios, 2005; Faske and Hurd, 2015; Vang et al., 2016). Chemicals can also be very costly, especially with the added requirements for nematicides such as plastic sheeting and chisels (Chitwood, 2003; Ntalli and Caboni, 2012; Faske and Hurd, 2015). Of more importance, nematicides can potentially be extremely harmful to the environment especially if not applied properly (Chitwood, 2003; Agrios, 2005; Ntalli and Caboni, 2012; Faske and Hurd, 2015). Available nematicides have decreased over time, with only a few available today, such as Fluopyram or 1,3-dichloropropene (Chitwood, 2003; Ntalli and Caboni, 2012; Faske and Hurd, 2015; Ebone et al., 2019). It can be even more difficult to find a nematicide that is labeled for a particular crop as not all nematicides are labeled for use on every crop (Chitwood, 2003; Ntalli and Caboni, 2012; Faske and Hurd, 2015). Regulatory interest of pesticides increased when the nematicides aldicarb and 1,2-dibromo-3-chloropropane (DBCP) were detected in groundwater (Chitwood, 2003; Ntalli and Caboni, 2012; Ebone et al., 2019). In 1984, all products containing ethylene dibromide (EDB) were banned (Chitwood, 2003; Ebone et al., 2019). In 1992, methyl bromide was removed from

the market because of correlations to depleting the ozone (Chitwood, 2003; Ntalli and Caboni, 2012). The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) added an amendment in 1988 that required registration of all pesticides to be reviewed (Chitwood, 2003; Ebone et al., 2019). Since then, available nematicides have declined due to organizations that deemed certain pesticides as harmful risks to humans and the environment (Chitwood, 2003; Agrios, 2005; Ntalli and Caboni, 2012; Faske and Hurd, 2015; Ebone et al., 2019). In addition, economic cost of research and registration of a new nematicide is extremely high and is often the first obstacle in developing a new product as it can take many years to produce a viable product (Chitwood, 2003; Agrios, 2005; Ebone et al., 2019). However, since nematodes continue to cause economic crop damage, a new generation of non-fumigant nematicides such as fluopyram, fluensulfone, and spirotetramat is emerging that have reduced mammalian toxicity and fewer harmful environmental effects (Faske and Hurd, 2015; Morris et al., 2016; Vang et al., 2016; Ebone et al., 2019). Many integrated pest management plans today include the use of a pesticide in combination with a cultural practice (Faske and Hurd, 2015; Vang et al., 2016). An integrated pest management plan that includes a combination of control methods with nematicides may be the most effective option to control nematodes (Chitwood, 2003; Faske and Hurd, 2015; Vang et al., 2016).

Cultural practices may be sufficient, or the only option, to use as a method to try to reduce nematode populations below economic damage thresholds (Agrios, 2005). One of the most common and effective cultural practices, depending upon the nematode and pathogen species present, is crop rotation (Agrios, 2005; Meadows et al, 2018). Rotation to a poor or non-host for at least one year is recommended to reduce nematode populations below a damaging threshold (Meadows et al., 2018). Nematode eggs will hatch and die from a lack of a proper host,

and the overall nematode (and other pathogen) population density will decrease (Sasser and Carter, 1985; Agrios, 2005). Crop rotation is often done by farmers regardless of a pathogen issue, as different types of crops require a specific season or the market for selling is more profitable for certain crops (Agrios, 2005; Meadows et al., 2018). However, rotation can be difficult when nematode species have an extremely wide range of host plant species (such as RKN), and the available non-host crops may be impractical or not economical to grow (Sasser and Carter, 1985). Crop rotation is useful when dealing with plant-parasitic nematodes with known non-hosts. For example, soybean cyst nematode (*Heterodera glycines*) can be controlled by planting from susceptible soybean to non-hosts such as cotton, corn, or grain sorghum (Niblack and Chen, 2004). A study found that planting soybean after one to two years following non-host crop rotation increased yield by up to 40% when compared to planting soybean following a soybean monoculture (Niblack and Chen, 2004; Koenning et al., 1993).

Besides sanitation, clean planting stock, and crop rotation, other cultural practices that can help control nematodes include fallowing soil, soil solarization, steaming, and flooding (Stover, 1979; Cadet et al., 2003; Kokalis-Burelle et al., 2016). Farmers can choose to fallow soils in order to help reduce nematode populations; fallow fields must be maintained weed-free (potential hosts), are prone to erosion, and provide no crop income to the farmer (Cadet et al., 2003). Soil solarization requires covering rows with plastic to retain heat and rarely reach killing temperatures at depths below 15 cm where nematodes can colonize the root system (Meadows et al., 2018). Both steaming and flooding require very specialized equipment or soil and water conditions for flooding, and flooding may result in accumulation of other types of diseases that proliferate in wet conditions (Stover, 1979; Agrios, 2005). Thus, fallowing soil, soil solarization,

steaming, and flooding are used only in very rare and specific circumstances to reduce nematode populations (Stover, 1979; Cadet et al., 2003; Agrios, 2005; Kokalis-Burelle et al., 2016).

A number of natural enemies of nematodes have evolved that may be utilized for biological control of nematode populations, especially within soil. The most common biological agents used to control nematodes are fungi and bacteria (Agrios, 2005). Nematophagous fungi such as *Monacrosporium* spp. or *Arthrobotrys* spp. can use sticky mycelial to capture nematodes (Nordbring-Hertz et al., 2006). *Purpureocillium lilacinus* is a fungus that parasitizes nematode eggs (Wilson and Jackson, 2013). A study was conducted that found both fungi, *Hirsutella rhossiliensis* and *Verticillium chlamydosporium,* were able to reduce *Meloidogyne hapla* populations and reduce their ability to enter host roots (Vianene and Abawi, 2000). Bacteria can also parasitize nematodes, with the most common being *Pasteuria penetrans*; *Pasteuria penetrans* can attach and penetrate the cuticle of juvenile nematodes, which eventually kills the juveniles (Wilson and Jackson, 2013). Biological controls have been shown to have positive results in controlling nematode populations. Biocontrols offer the potential to non-chemically affect nematodes in a system that could offer long term management (Agrios, 2005; Nordbring-Hertz et al., 2006; Wilson and Jackson, 2013). Several biological products are available on the market to aid in nematode control. For example, Poncho VOTiVO (Bayer CropScience) is a biological seed treatment that controls nematodes by using the bacteria *Bacillus firmus*. The bacteria colonize around the plant's roots, creating a biological barrier of protection, and has shown to increase yield (Wilson and Jackson, 2013). Marrone has a bionematicide, named Majestene, that utilizes *Burkholderia rinojensis* to control RKN such as *M. chitwoodii* (Marrone, 2018; Williamson and Gleason, 2003). Results have shown that Marrone can increase yield on crops that are under pressure from plant-parasitic nematodes and can even provide better efficacy

than nematicides, such as potato yield was increased in Majestene treated plots compared to the nematicide Vydate treated crops (Marrone, 2018; Williamson and Gleason, 2003). However, it can be difficult to establish biological agents in the field to survive extended periods of time to maintain the efficacy of the biological control agent without the environment being a factor (Lambert and Bekal, 2002).

Genetic resistance in host plants is a method of nematode management that can be environmentally friendly and economically efficient (Sasser and Carter, 1985; Trudgill, 1991; Lambert and Bekal, 2002). Plants are considered resistant to nematodes when nematode reproduction levels are reduced to little or no reproduction (Sasser and Carter, 1985). Tolerance is the ability of the host genotype to support nematode infection and reproduction without jeopardizing the yield, until population levels rise to damaging thresholds (Sasser and Carter, 1985; Trudgill, 1991). Plant resistance to pathogen infection stems from either preformed or induced mechanisms (Holbein et al., 2016). Preformed resistance is expressed at all times, even when uninfected (Agrios, 2005). Preformed mechanisms include physical barriers such as cell wall lignification and fortification and chemical barriers such as deterrent root exudates or toxin accumulation in tissues (Dalmasso et al., 1992; Holbein et al., 2016). Induced resistance occurs in response to nematode infection, typically involving a gene in the host plant that confers a response to nematode infection to block or suppress the nematode activity (Williamson and Hussey, 1996). Induced resistance responses can include localized expression and accumulation of reactive oxygen species, phytoalexins, and pathogenesis-related (PR) proteins (Yang et al., 2018). Resistance can occur in varying levels that are controlled by one gene or multiple genes (Chitwood, 2003; Yang et al., 2018). One of the most commonly recognized resistance genes conferring resistance against the RKN species *M. incognita, M. javanica,* and *M. arenaria* is the

Mi gene in tomato (Vos et al., 1998). The area of roots in which nematodes are invading is targeted by a localized hypersensitive response in which rapid necrosis occurs around the area of infection (Chitwood, 2003). Results have shown that the hypersensitive response occurs about 12 hours after inoculation of roots by juvenile RKN, which suggests that the response occurs when nematodes attempt to initiate giant cells for feeding (Vos et al., 1998). Another example of resistance occurs in potato involving the *H1* gene and *Globodera rostochiensis*, the potato cyst nematode (PCN) (Finkers-Tomczak et al., 2011). A hypersensitive response occurs as the tissue around the PCN feeding site (syncytium) becomes necrotic and collapses (Finkers-Tomczak et al, 2011). Nematode reproduction is greatly reduced, and any PCN that develop typically turn into non-feeding males due to the lack of nutrition with few females available to lay eggs (Chitwood, 2003; Finkers-Tomczak et al., 2011).

Plant breeders must identify genotypes that have resistance and cross nematode resistance genes into cultivated plants in attempts to transfer resistance into desirable commercial cultivars. There are numerous benefits to this method of controlling nematodes. It is relatively inexpensive for growers to implement resistant plants in their field compared to other methods such as applying nematicides (Lambert and Bekal, 2002; Dong et al., 2007). Natural sources of resistance genes provide a non-chemical method to manage pathogens, which may limit environmental or human impacts (Lambert and Bekal, 2002). In times of intense scrutiny regarding the use of pesticides and the limited available nematicides to growers, a non-chemical management tool can be important. This technique can have drawbacks however, as it takes years to screen to identify resistance and to then breed the resistance into a commercial variety (Lambert and Bekal, 2002). Some resistant cultivars may have a reduced yield in the absence of a pathogen (yield drag), have less desirable agronomic or horticultural traits such as taste, or are

unable to grow well in existing environmental conditions (Sasser and Carter, 1985; Lambert and Bekal, 2002; Agrios, 2005). In addition, not all species of crop plants have available known resistance genes, therefore other management tools must be used for farmers that grow those plants lacking genetic resistance (Lambert and Bekal, 2002). Even if a cultivar has resistance and a desirable final product to market, nematodes have the potential to overcome resistance in time (Lambert and Bekal, 2002). Planting the same cultivar can lead to the development of heritable virulence in the nematode population and eventually leads to the loss in utility of resistance (Sasser and Carter, 1985; Lambert and Bekal, 2002). Thus, implementing an integrated management plan that includes genetic host resistance alternated with other management strategies is often the best practice (Lambert and Bekal, 2002; Agrios, 2005).

Plant-Parasitic Species in North Carolina

The agricultural industry is extremely important in North Carolina. Food, ornamentals, fibers, and forestry industries account for 17% of North Carolina's state income, or about $84 billion (Ye, 2018; USDA, 2019). An estimated 8.2 million out of 12.5 million hectares of land in North Carolina is utilized for food and fiber production (Ye, 2018; USDA, 2019). RKNs pose a serious threat to turf grasses, ornamentals, fruit trees, vegetables, and field crops, which highlight the importance of nematode management (Sasser and Carter, 1985). North Carolina is the nation's leading producer in tobacco and sweetpotato production (USDA, 2019), which are high value crops that are potential hosts to plant-parasitic nematodes, including several RKN species (Sasser et al, 1983; Ye, 2018). In North Carolina, there are about 82 plant-parasitic nematodes that include RKN (*Meloidogyne* spp.), cyst (*Heterodera* spp.), reniform (*Rotylenchulus* spp.), sting (*Belonolaimus* spp.), lesion (*Pratylenchus* spp.), and lance (*Hoploaimus* spp.) nematode

species to name a few (Sasser et al., 1983; Ye, 2018). However, the most commonly identified plant-parasitic nematodes in North Carolina are the RKNs (*Meloidogyne* spp.) (Sasser and Carter, 1985; Meadows, 2018; Ye, 2018); the most common *Meloidogyne* species include *M. incognita, M. arenaria, M. hapla*, and *M. javanica* (Sasser and Carter, 1985; Ye, 2018). There are currently 11 *Meloidogyne* species recorded in North Carolina, which among them include the highly aggressive and newly emerging guava root-knot nematode, *M. enterolobii* (Ye, 2018).

Meloidogyne enterolobii

Meloidogyne enterolobii is a very aggressive species of RKN. This nematode was first described in 1983 from roots of pacara earpod trees (*Enterolobium contortisiliquum*) in China (Yang and Eisenback, 1983). In 1988, an RKN species called *M. mayaguensis* was described from roots of eggplant (*Solanum melongena*) in Puerto Rico (Rammah and Hirschmann, 1988). In 2004, it was determined that *M. mayaguensis* was a synonymous species with *M. enterolobii* and has been considered as *M. enterolobii* since then (Xu et al., 2004). *Meloidogyne enterolobii* is commonly referred to as the guava RKN due to its prevalence in Brazil causing large economic losses in guava (Hare, 2019). This nematode is found in many countries in Asia, Africa, and South America (Anonymous, 2014; Gao et al., 2014; Subbotin, 2019). It has recently been reported in North America, with reports in Florida in 2004 (Brito et al., 2004), North Carolina in 2013 (Ye et al., 2013), South Carolina in 2019 (Rutter et al., 2019), and Louisiana in 2019 (Hare, 2019).

Like other species of *Meloidogyne, M. enterolobii* has a very wide plant host range (Subbotin, 2019). Although there is still uncertainty of the full defined host list, some of the hosts include important crops such as sweetpotato, soybean, tobacco, cotton, tomato, peppers,

watermelon, coffee, and guava to name a few (Anonymous, 2014; Gao et al., 2014; Chiamolera et al., 2018; Subbotin, 2019). In addition, this species can infect many weed species that cause management to be more difficult (European and Mediterranean Plant Protection Organization, 2009). *Meloidogyne enterolobii* is of significant concern for its ability to overcome known RKN resistant genes in plants including *Mi-1* in tomatoes, *Mh* gene in potato, *Mir1* gene in soybean, *Tabasco* gene in sweet pepper, and *N*-carrying peppers are all susceptible to *M. enterolobii* (Kiewnick et al., 2009, Anonymous, 2014). This ability to overcome these resistance genes displays the high virulence and aggressiveness of *M. enterolobii* (Kiewnick et al., 2009). The list of poor or non-hosts is very limited and still mostly unknown; however, the list includes peanut, grapefruit, garlic, corn and sour orange (Castagnone-Sereno, 2011; Anonymous, 2014). This limited list of known non-host crops for potential rotations highlights the need for discovery of more crop genotypes with resistance to *M. enterolobii.*

Like other RKN, *M. enterolobii* causes characteristic root galls and reduces crop yield (Agrios, 2005; Anonymous, 2014). Galls seem to be larger in size when caused by an *M. enterolobii* infection compared to other RKN species, however there is currently no quantitative evidence to support this claim. A study by Cetinta et al. (2007) compared the yield differences of tomato based on the infecting nematode species. They found that *M. arenaria* infection caused 42% crop loss when compared to a control, *M. javanica* and *M. incognita* caused 46% loss, and *M. enterolobii* caused 65% crop loss (Cetintas et al., 2007). These results, along with the nematode's ability to overcome other root-knot resistant genes, help show the aggressive nature of this nematode and the need for research into its distribution and potential management strategies.

In 2010, *M. enterolobii* was added to the European and Mediterranean Plant Protection Organization (EPPO) A2 alert list (European and Mediterranean Plant Protection Organization. 2009; Subbotin, 2019). This organization helps to monitor transportation of plant material to ensure stock is not infected. The EPPO has already intercepted plant stock containing *M. enterolobii* being shipped from Asia, South America, and Africa to countries like the Netherlands and Germany (EPPO Report, 2009). It has not been found to exist outside greenhouses in northern European countries where it has been detected (EPPO Report, 2009). *M. enterolobii* is a tropical to sub-tropical species, which may explain why it has not been found naturally in northern countries where the temperatures are not as optimal (Anonymous, 2014). However, all infected plant stock should not be transported regardless of its destination. In addition, the growing concern and threat of *M. enterolobii* has led to quarantines in North Carolina, Louisiana, Arkansas, and Mississippi (Weimin Ye, personal communication). The North Carolina quarantine (http://www.ncagr.gov/PLANTINDUSTRY/plant/disease/grkn.htm) restricts movement of sweetpotato planting material, as only certified and tested material is allowed to be transported outside of state lines in order to help reduce the chances of *M. enterolobii* being introduced to new areas.

Identification of *Meloidogyne enterolobii*

Traditional identifications of species of *Meloidogyne* have relied upon microscopy to compare morphology with perineal patterns of females as a classic identification method for RKN (Sasser and Carter, 1985; Karssen and Aelst, 2000). Perineal patterns are the cuticular annulations and striations that surround the vulva-anus area and tail terminus of adult female RKN that provide a unique pattern that can be species specific (Karssen and Aelst, 2000). The

perineal pattern of *M. enterolobii* is very similar to that of *M. incognita*, as intraspecific variations are closely related, and often cannot distinguish between the two with accuracy (Karssen and Aelst, 2000). When only RKN J2 can be recovered from a sample, the use of morphology to distinguish species becomes especially challenging and more subjective to interpretation and requires extensive training (Karssen and Aelst, 2000).

Advances in molecular biology have yielded contemporary DNA analysis as an objective and reproducible means to identify taxa, including nematodes (Tigano et al., 2010). When small sample sizes (like nematodes) are available, the polymerase chain reaction (PCR) allows for amplification of unique and specific sequences from DNA template that correlates to a specific sequence in the nematode that can be used to identify to the species level (Karssen and Aelst, 2000). PCR primers to distinguish species of *Meloidogyne* such as *M. incognita* and *M. javanica* were among the first molecular diagnostic assays developed for plant parasitic nematodes (Karssen and Aelst, 2000). The development of PCR primers to identify *M. enterolobii* is relatively recent (Long et al., 2006; Tigano et al., 2010), and the genome of *M. enterolobii* was recently sequenced (Koutsovoulos et al., 2019). Primers that existed before the full genome sequence was discovered were based on the draft genome, or the partial genome (Tigano et al., 2010). Currently, there are a few *M. enterolobii* specific primers. Tigano et al. (2010) created a sequence characterized amplified region (SCAR) primer that has been proven to be specific to *M. enterolobii*. Primer MK7-F (5′-GATCAGAGGCGGGCGCATTGCGA-3′) and MK7-R (5′-CGAACTCGCTCGAACTCGAC-3′) were confirmed to be accurate on 16 different *M. enterolobii* isolates and not on other species (Tigano et al., 2010). Another common primer specific to *M. enterolobii* is Me-F/Me-R (AACTTTTGTGAAAGTGCCGCTG/TCAGTTCAGGCAGGATCAACC) (Long et al., 2006).

While DNA analyses are useful for nematode species identification, no molecular tests exist to compare intraspecific differences within a plant parasitic nematode species. Species can develop different lineages that alter their virulence on potential hosts (Sasser and Carter, 1985; Sasser et al., 1983; Robertson et al., 2009). For example, *M. javanica* race 2 reproduces on pepper, and race 3 reproduces on peanut and not pepper (Robertson et al., 2009). These results are based on host differential tests, or phenotypic host assessments (Sasser et al., 1983). Different physiological races show that virulence can vary depending on the population of nematode species (Sasser and Carter, 1985). A study in Brazil was conducted to assess potential differences in populations of *M. enterolobii* based on isoenzyme phenotype. The group found that 16 different isolates of *M. enterolobii* had a low level of diversity (Tigano et al., 2010). These 16 isolates came from various countries such in Brazil, Puerto Rico, Congo, Costa Rica, and Guatemala which the study found all to be homogenous species (Tigano et al., 2010). Another study conducted found that five populations of *M. enterolobii* from Africa were all genetically homogenous based on nucleotide sequencing and phylogenetic analysis of the intergenic spacer (IGS) fragment (Onkendi and Moleleki, 2013). DNA analyses may not indicate virulence, therefore, it is important to identify differences in population virulence, based on the differential host test, to accurately describe populations that can affect nematode management long-term.

Plant resistance to *Meloidogyne enterolobii*

A study was conducted in which seven guava rootstocks were assessed for resistance to *M. enterolobii* (Chiamolera et al., 2018). They found that three out of seven guava genotypes were resistant to the nematode species. A study was conducted that found a gene (*Ma*) from Myrobalan plum (*Prunus cerasifera*) confers resistance to many RKN species including *M.*

enterolobii (Claverie et al., 2011). Since *M. enterolobii* can overcome common RKN resistance genes (Kiewnick et al., 2009), it is vital to identify genotypes with resistance to this species of nematode, especially in high value crops that are dominantly grown in areas that *M. enterolobii* is located. For example, while sweetpotato genotypes have been identified with resistance to the major RKN species (Cervantes-Flores et al., 2002), it is unknown if these or other sweetpotato genotypes are also resistant to *M. enterolobii.* Host resistance represents an efficient, reliable, and environmentally non-toxic method to manage RKNs (Lambert and Bekal, 2002); therefore, it is vital to identify host resistance to *M. enterolobii*.

Meloidogyne enterolobii in North Carolina

In December 2011, the NCDA&CS Agronomic Division Nematode Assay Lab analyzed roots of stunted cotton plants from Wayne County, North Carolina from two separate fields (Ye et al., 2013). The lab performed a PCR test to identify the species of nematode that caused the extreme galls on the cotton roots. *Meloidogyne enterolobii* was confirmed as the causal species, which was the first confirmed detection of *M. enterolobii* infestation within field soils in North Carolina. In August 2012, the NCDA lab received damaged soybean plants from Wayne and Johnston county. These plants were also confirmed by DNA analyses as infected by *M. enterolobii* (Ye et al., 2013). Since the NCDA Nematode Assay Lab does not routinely identify RKN to species level unless requested or warranted, the actual distribution of *M. enterolobii* in North Carolina is of concern since it has many host crop species and has been in the state for a number of years. How *M. enterolobii* was introduced into North Carolina remains unknown, which raises the importance of proper identification of the nematode and knowing its current distribution in order to manage and prevent it from spreading into new areas. Determining if all

North Carolina *M. enterolobii* populations stemmed from the same source or if populations have different lineages can be vital in terms of resistance and management if populations differ in virulence.

Threat of *Meloidogyne enterolobii* to crops in North Carolina

At the initiation of this research in Fall 2017, *M. enterolobii* had been identified in a number of North Carolina crops in Wayne, Johnston, Wilson, and Columbus counties (Weimin Ye, personal communication). Of primary concern were a number of reports of *M. enterolobii* infection of sweetpotatoes grown in these counties (Ye, 2018).

North Carolina is the nation's largest sweetpotato producing state (Ye, 2018). Not only does *M. enterolobii* infect the fibrous roots and reduce plant vigor and yield, the nematode also infects the underground storage roots that are the marketable sweetpotato product (Rutter et al., 2019). In 2014, An estimate of 73,000 acres of sweetpotatoes, or roughly $354 million in gross farm value, was grown which represents more than half of the sweetpotato production in the United States (Barkley et al., 2017). The sweetpotato breeding program at North Carolina State University developed and released 'Covington' sweetpotato in 2008 (Yencho et al., 2008). 'Covington' is the leading sweetpotato variety grown in North Carolina due to its marketable qualities, success in the field, and its resistance to pathogens such as *M. incognita* (Yencho et al., 2008). 'Covington' represents about 88% of sweetpotatoes grown in North Carolina (Barkley et al., 2017). The recent identifications of *M. enterolobii* infection of sweetpotato in North Carolina have been in 'Covington' and has been independently confirmed that 'Covington' is susceptible to *M. enterolobii* (Rutter et al., 2019). It is unknown if resistance to *M. enterolobii* exists in other

sweetpotato genotypes or cultivars with reported resistance to RKN species (Cervantes-Flores et al., 2002; Chapter 3)

Given the threat of *M. enterolobii* to sweetpotato production in North Carolina, the research presented here included investigations of the distribution of *M. enterolobii* on crops grown in eastern North Carolina, potential variability in virulence among selected North Carolina *M. enterolobii* populations, and greenhouse evaluations of selected sweetpotato genotypes for potential resistance to *M. enterolobii*.

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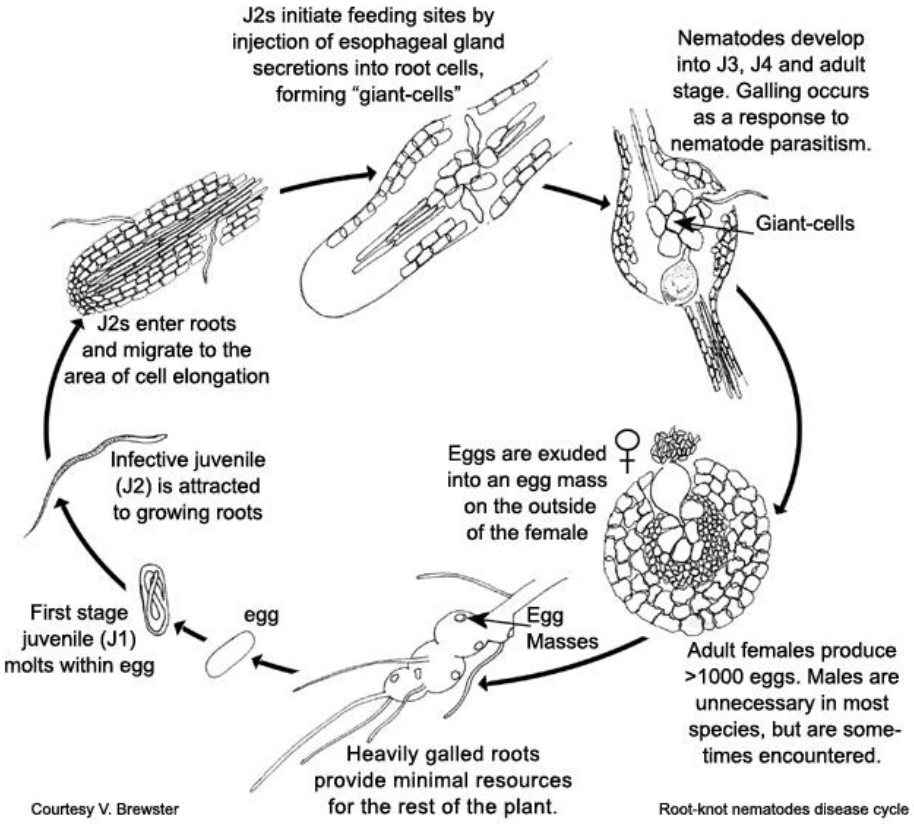

Figure 1. Life Cycle of the root-knot nematode, *Meloidogyne* species. (Reproduced from Mitkowski and Abawi, 2003; Dr. Rajendra Singh)

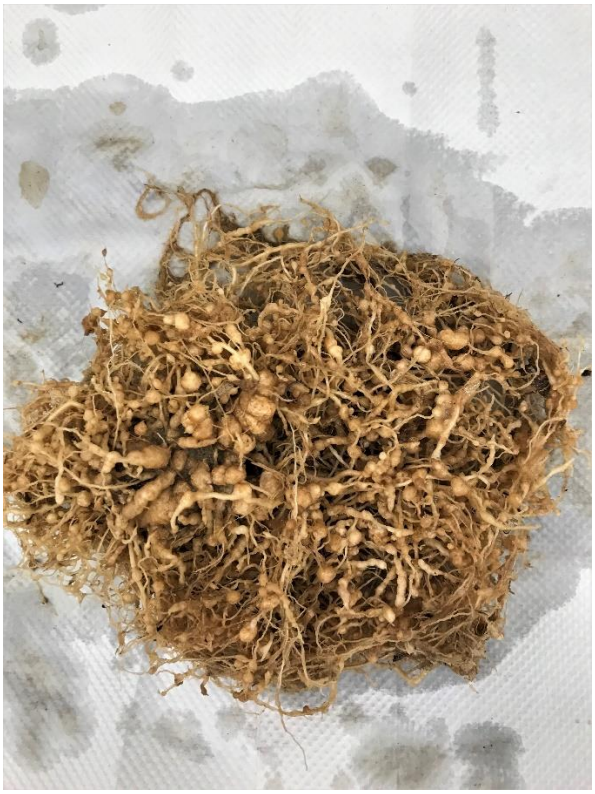

Figure 2. Characteristic galls on ‘Rutgers’ tomato roots (*Lycopersicon lycopersicum* L) infected by *Meloidogyne enterolobii.*

Figure 3. Sweetpotato storage root infected with *Meloidogyne enterolobii.*

Distribution of *Meloidogyne enterolobii* in Eastern North Carolina and Comparison of four isolates

Abstract. The root-knot nematode (RKN) species, *Meloidogyne enterolobii*, is a particularly aggressive pathogen with limited distribution. In 2011, *M. enterolobii* was identified on field crops in North Carolina for the first time. In collaboration with the North Carolina Department of Agriculture and Consumer Services (NCDA&CS) Nematode Assay lab, RKN positive samples from the eastern-half of North Carolina were collected and analyzed for the presence of *M. enterolobii* using nematode DNA in polymerase chain reaction (PCR). PCR primers that identify the predominant RKN species in North Carolina, *M. incognita, M. arenaria, M. javanica,* and *M. hapla*, as well as primers that identify *M. enterolobii*, were used to analyze DNA from 203 RKN-positive samples representing a variety of field and vegetable crops grown in counties in the eastern half of North Carolina. New detections of *M. enterolobii* were found in Nash, Greene, Sampson, and Harnett counties in addition to the previously identified locations in Johnston, Wayne, Columbus, and Wilson counties. Four isolates of *M. enterolobii* populations were collected from soybean and sweetpotato crops in Johnston, Greene, and Wilson counties and reared on 'Rutgers' tomato plants in the greenhouse. Potential differences among the four populations were analyzed based on phenotypic assessments on six sweetpotato genotypes in replicated greenhouse tests. 'Tanzania', 'Centennial', 'Murasaki-29', and 'NC607-16' were sweetpotato genotypes used that conveyed resistance to *M. enterolobii*, and 'Covington' and 'NCDM04-0001' were sweetpotato genotypes included that are susceptible to *M. enterolobii.* There were no significant differences in gall ratings or eggs per gram of root among the four *M. enterolobii* isolates on the six sweetpotato genotypes that were assayed.

Introduction

Root-knot nematodes (RKN, *Meloidogyne* spp.) cause the most damage on economic crops compared to any other nematode (Jones et al., 2013). There are approximately 100 described species of *Meloidogyne* (Mitkowski and Abawi, 2003). They parasitize a wide range of plants, with more than 2,000 plant species known to be suitable hosts (Sasser and Freckman, 1987). RKN live in the soil and infect host plants in large numbers, causing root damage that leads to a reduction in plant vigor and yield (Ye, 2018). Depending on the plant species, RKN can impair the value, storage, and marketability of the harvested crop (Anonymous, 2014). The emergence of the aggressive RKN species *Meloidogyne enterolobii* is a threat to crop production (Castagnone-Sereno, 2012). *M. enterolobii* was first described in China in 1983 (Yang and Eisenback, 1983) on pacara earpod tree (*Enterolobium contortisiliquum)*. It has been detected in countries in Africa, Asia, South America, and North America (Yang and Eisenback, 1983; Brito et al., 2007; Anonymous, 2014; Subbotin, 2019). In the United States, this species has been reported in Florida (Brito et al., 2004), North Carolina (Ye et al., 2013), Louisiana (Hare, 2019), and South Carolina (Rutter et al., 2019). Of particular concern is the ability of *M. enterolobii* to infect and reproduce on hosts with RKN resistance genes such as *Mi-1* in tomato and *N*-gene peppers (Kiewnick et al., 2009: Castagnone-Sereno, 2012). However, in order to properly manage *M. enterolobii,* we need to know the full extent of where this species is located. In North Carolina, the state's most valuable crops, including sweetpotato and tobacco, are at risk of infection by *M. enterolobii* (Ye, 2018) and the extent of its distribution in the state is largely unknown.

The first identification of *M. enterolobii* infection of crops in North Carolina (Ye et al., 2013) was from damaged cotton plants (*Gossypium hirsutum* L. cvs. PHY 375 WR and PHY 565

WR) that were collected from two separate fields in Wayne County by the North Carolina Department of Agriculture & Consumer Services (NCDA&CS) Nematode Assay Lab. Species identification of DNA through polymerase chain reaction (PCR) confirmed the RKN species as *Meloidogyne enterolobii* (Ye et al., 2013). In 2012, the NCDA&CS received galled soybean (*Glycine max* (L.) Merr. cv. 7732) plants from Wayne and Johnston County which were also confirmed to be infected by *M. enterolobii* through DNA analysis (Ye et al., 2013).

At the initiation of the survey reported in this chapter, *M. enterolobii* had been reported in Columbus, Johnston, Wayne, and Wilson counties in North Carolina in cotton and soybean, and in tobacco and vegetable crops including cucurbits and sweetpotatoes in 2017 (Ye, 2018; Personal communication with NCDA&CS). In this study, we analyzed RKN samples from a range of crop species (Table 1) in counties in the eastern half of North Carolina to more comprehensively determine the potential geographic distribution of *M. enterolobii* in this region of the state.

Morphological identification of RKN species requires considerable training and may be prone to subjectivity without the requisite expertise (Karssen and Aelst, 2000). For example, perineal patterns between *M. incognita* and *M. enterolobii* are difficult to distinguish with accuracy (Karssen and Aelst, 2000). Analysis of DNA sequences that are species-specific using end-point polymerase chain reaction (PCR) provides a method to identify species that can be more objective and accurate (Tigano et al., 2010; Subbotin, 2019). Multiple investigations and improved technology have led to the discovery of DNA primers sets that distinguish selected species of RKN (Table 2), including *M. enterolobii* (Zijlstra, 2000; Long et al., 2006; Tigano et al., 2010).

In addition to the RKN survey in eastern North Carolina, four populations of *M. enterolobii* derived from different geographic samples submitted to the NCDA&CS Nematode Assay Lab were collected and used as inoculum to start independent greenhouse cultures. Since nematodes can develop different genetic lineages within a species with altered virulence on potential hosts, a study was conducted to assess potential differences in virulence among the four *M. enterolobii* populations collected in North Carolina (Sasser and Carter, 1985). For example, *M. javanica* race (biotype) 2 reproduces on pepper, and race 3 reproduces on peanut and not pepper (Robertson et al., 2009). These results are based on phenotypic assessments in plant host differential tests. Different biotypes show that virulence can vary depending on the population within a nematode species (Sasser and Carter, 1985), however, not much is known about possible biotypes within *M. enterolobii.* Using DNA analyses, a study in Brazil found that sixteen different isolates of *M. enterolobii* had a low level of genetic diversity (Tigano et al., 2010). These sixteen isolates came from various regions such in Brazil, Puerto Rico, Congo, Costa Rica, and Guatemala which the study found all to be genetically homogeneous species (Tigano et al., 2010). Similarly, five populations of *M. enterolobii* from Africa were all determined to be genetically homogeneous (Onkendi and Moleleki, 2013). Since potential variability in virulence within populations of *M. enterolobii* can be critical to effective crop management, the study here focused on the ability of the four North Carolina populations to infect a selected set of sweetpotato host genotypes in order to detect potential differences in virulence among the *M. enterolobii* populations on sweetpotato.

In this study, RKN samples from the eastern-half of North Carolina were identified for the presence of *M. enterolobii* in order to provide an understanding of the extent of this RKN's geographic distribution in North Carolina. It is hypothesized that *M. enterolobii* is located in

more North Carolina counties than those previously identified. In addition, four North Carolina isolates of *M. enterolobii* were assessed for potential differences in virulence based on a sweetpotato host test. Species identification can have impacts on management practices; therefore, it is vital to identify the distribution of *M. enterolobii* and potential differences in population virulence.

Materials and Methods

Survey of RKN species in eastern North Carolina

Root and/or soil samples (n=203, Fig. 1) from various crops (Table 1) were collected from the eastern-half of North Carolina that were submitted to the NCDA&CS Nematode Assay Lab by growers between October 2017-March 2019 for analysis of potential nematode infestations in their fields. The Nematode Assay Lab used inverted microscopes to count and identify all plant-parasitic nematodes to the genus level in each sample. Samples selected from a range of eastern North Carolina counties and crops that contained RKN were chosen to conduct RKN species identification by PCR in this survey. Figure 1 shows the total number of RKN samples collected per county. A total of 203 samples were analyzed for RKN species identification and the potential presence of *M. enterolobii*.

To identify RKN species from root samples, roots were placed under a dissecting microscope and *Meloidogyne* females were dissected from galls. A single female was put in 15-µl of TE buffer and macerated using tweezers to release and suspend the DNA. For samples that only contained RKN stage-two juveniles (J2), the J2 in water were transferred by pipette to a slide under an inverted compound microscope and the pipette tip was used to crush the nematode to release DNA in the water droplet (Powers et al, 2005). The J2 DNA in water was combined

with TE buffer to a volume of 15-µl. RKN DNA samples in TE were stored at -80°C prior to PCR analysis.

DNA primers were used in PCR to differentiate unknown species of RKN found in each respective sample. The primers used in this study were specific to *M. incognita, M. arenaria, M. javanica, M. hapla,* and *M. enterolobii* (Table 2). Two different primers were used to identify for the presence of *M. enterolobii.* Amplifications were performed by using 20-µL volumes containing 1-µL of DNA from the RKN sample, 0.5-µL forward primer, 0.5-µL reverse primer, 8-µL water, and 10-µL of *taq* polymerase. The temperature profile for all PCR reactions was 95°C for 2 minutes, followed by 40 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 45 seconds, and a final extension stage at 72°C for 10 minutes. The PCR products were separated by electrophoresis in a 2% agarose gel, and the size of the DNA fragments were compared to those predicted by the primer sets in Table 2 that differentiate RKN species (Figure 4).

Virulence of Four NC Populations of *M. enterolobii*

Four different sources of *M. enterolobii* (Figure 3) were used to develop cultures that were maintained in the greenhouse. One population of *M. enterolobii,* termed ’18-5126’, came from infested soil of a soybean field rotated with sweetpotato in Johnston County, North Carolina. The other three populations were isolated from a sweetpotato storage root: ’18-6908’ population from Wilson County, ’18-8260’ population from Greene County, and ‘ent11’ from Greene County (Figure 3). All four sources were kept separate and cultured on roots of ‘Rutgers’ tomato plants (*Lycopersicon lycopersicum*) in the greenhouse. All four sources were initially identified by PCR as *M. enterolobii* by the NCDA Nematode Assay Lab. After increasing the

nematode populations in the greenhouse, identification was performed by PCR to confirm that only *M. enterolobii* was present in all four cultures.

Potential differences in virulence among nematode populations were analyzed through phenotypic assessments on selected sweetpotato genotypes. The six sweetpotato genotypes chosen were 'Centennial', 'Tanzania', 'Murasaki-29', and 'NC607-16' which are all considered resistant to *M. enterolobii,* and 'Covington' and 'NCDM04-0001' which are both susceptible to *M. enterolobii* (Chapter 3).

Twelve to fifteen-cm-long sweetpotato cuttings (slips) were planted in four-inch-deep plastic pots containing a 3:1 sand soil mixture (88.9% sand, 8.2% silt, 2.8% clay) in the greenhouse. After two weeks of root growth, each plant was inoculated with about 10,000 eggs of *M. enterolobii* from each respective culture. Four replicates of each *M. enterolobii* population on each sweetpotato genotype were evaluated.

Plants were evaluated 60 days post inoculation with *M. enterolobii*. All plants were cut at the crown to isolate the roots. Each root system was rinsed of soil and the fresh weight was recorded. Visual gall ratings were conducted based on the percent of the entire root system that had galls. RKN eggs were then extracted from each whole root system using the NaOCl method described by Hussey and Barker (1973). Aliquots of eggs from each root system were counted using an inverted microscope. The total egg count was divided by the weight of the root to provide the number of eggs per gram of root. Gall ratings and eggs per gram of root were used to compare *M. enterolobii* populations to assess possible phenotypic differences among the four populations. Data were analyzed using statistical analysis software SAS (SAS Institute, Cary, NC) using the generalized linear model (PROC GLM). All data were transformed by log(x+1) to

standardize variance for statistical analyses due to the variation between resistant and susceptible genotypes. Transformed means were separated using Fisher's *t* test LSD procedure (alpha=0.05).

Results

The majority of *Meloidogyne* species detected in 203 RKN-positive samples from eastern North Carolina contained *M incognita* or *M. arenaria*. *M. hapla* and *M. javanica* were also detected, but in much fewer samples than *M. incognita* or *M. arenaria*: 65 out of 203 samples (32%) were identified as *M. incognita,* 49 out of 203 samples (24%) were identified as *M. arenaria*, 26 out of 203 samples (13%) were identified as *M. javanica,* and 19 out of 203 samples (9%) were identified as *M. hapla. M. enterolobii* was detected at a much lower rate than the other *Meloidogyne* species, as 12 out of 203 samples (6%) were identified, and was found in samples from Nash, Greene, Sampson, and Harnett counties in North Carolina that represent new counties detected with this species of RKN. Johnston, Wayne, and Wilson counties were also confirmed again as locations containing *M. enterolobii.* Columbus County was the only county previously reported to contain *M. enterolobii* that was not detected in the samples examined in this survey, as 0 out of a total 7 samples from this county contained *M. enterolobii* (Figure 4). The *M. enterolobii*-positive counties are all adjacent to each other in central North Carolina with the exception of Columbus County on the southern border with South Carolina (Figure 4). *M. enterolobii* was detected in only a small fraction of total samples from each *M. enterolobii*-positive county: 1 out of 2 samples from Harnett County, 1 out of 7 from Greene County, 1 out of 10 from Sampson County, 1 out of 9 from Wayne County, 3 out of 27 from Johnston County, 4 out of 19 from Nash County, and 1 out of 14 from Wilson County contained *M. enterolobii.*

Not every sample was identified to the species level, as 32 out of 203 samples (16%) were unable to confirm an RKN species using the primer sets in Table 2. The majority of samples came from fields of sweetpotato, tobacco, and soybean, but also included peanut, cotton, potato, and cucurbits (Table 1). A large portion of samples (61) came from unidentified or unreported crops, and 2 of those 61 samples were identified as *M. enterolobii*. *M. enterolobii* was identified in samples from sweetpotato, tobacco, and soybean, which were all known host crops of *M. enterolobii.* There was no trend in terms of RKN species and host crop. No RKN-positive samples from peanut or corn were identified that contain *M. enterolobii.*

The results of screening the four North Carolina isolates of *M. enterolobii* on six selected sweetpotato genotypes found all four isolates to be the homogeneous as no significant differences among the four isolates when comparing infection rates on each genotype were found (Figure 5). Values presented in Figure 5 represents the mean of non-transformed data. There were no significant differences between gall ratings ($P < .3240$) or eggs per gram of root ($P < .1317$) among the four isolates. Sweetpotato genotype was significant ($P < 0.001$) for gall ratings and eggs per gram of root. The sweetpotato genotype ‘Centennial’ had consistent gall ratings of 0% and eggs per gram of root among the four isolates. *M. enterolobii* isolate ’18-6908’ had a mean of 4.79 eggs per gram of root, ’18-8260’ averaged 3.33 eggs/g root, ‘ent11’ averaged 3.45 eggs/g root, and ’18-5126’ averaged 2.43 eggs/g root on the genotype ‘Centennial’. The genotypes ‘Murasaki-29’, ‘Tanzania’, and ‘NC607-16’, which are resistant to *M. enterolobii,* also had consistent gall ratings of 0% and eggs per gram of root among the four *M. enterolobii* populations. Susceptible sweetpotato genotype ‘Covington’ averaged 4435 eggs/g root for isolate ’18-6908’, ’18-8260’ averaged 5597 eggs/g root, ‘ent11’ at 5363 eggs/g root, and ’18-5126’ averaged 5494 eggs/g root. Eggs per gram of root and gall ratings were not significantly different

among the four isolates. The other susceptible sweetpotato genotype, 'NCDM04-0001' averaged 11866, 7676, 10622, and 7143 eggs per gram of root with *M. enterolobii* populations '18-6908', '18-8260', 'ent11', and '18-5126' respectively. Eggs per gram of root and gall ratings were not significantly different among the four isolates on the susceptible sweetpotato genotypes.

Discussion

Accurate identification of nematode species is vital to understand the extent of geographic distribution for the species and for proper management (Sasser and Carter, 1985; Cervantes-Flores et al., 2002). In North Carolina, the predominant species of RKN are *M. incognita, M. arenaria, M. javanica,* and *M. hapla* (Sasser and Carter, 1985). These species represent a threat to crop production, but research has provided resistant genotypes and non-hosts to control the damage caused by one or all of the four species listed (Cervantes-Flores et al., 2002; Dong et al., 2007; Yencho et al., 2008). However, the emergence of *M. enterolobii* in North Carolina is threatening crop production as the locations of the nematode and sources of crop resistance are emerging.

Prior to this study, *Meloidogyne enterolobii* had been detected in Columbus, Johnston, Wayne, and Wilson counties in North Carolina (Weimin Ye, NCDA&CS, personal communication; Ye et al., 2013). The survey presented here of 203 RKN-positive crop samples found *M. enterolobii* in Nash, Greene, Sampson, and Harnett counties, in addition to Johnston, Wayne, and Wilson counties. Although *M. enterolobii* was previously confirmed in Columbus County (Weimin Ye, NCDA&CS, personal communication), no *M. enterolobii*-positive samples were detected from Columbus County in the survey presented here.

Understanding the distribution of *M. enterolobii* is important. RKN have limited movement in the soil (Sasser and Carter, 1985), however, they can spread long distances through human error if proper precautions are not implemented (Lambert and Bekal, 2002). RKN can disseminate longer distances through infected soil, equipment, water, plants, seeds, and bulbs (Lambert and Bekal, 2002). Proper sanitation is required to prevent the spread of nematodes to unaffected fields (Sasser and Carter, 1985; Lambert and Bekal, 2002; Thiessen, 2018). Knowing the geographic range of *M. enterolobii* will help to limit the spread of this species as increased sanitation and awareness should be implemented in an *M. enterolobii* positive field. In addition, understanding the location of this RKN species may help to prevent the transportation of infected plant material. The European and Mediterranean Plant Protection Organization (EPPO) had intercepted plants that were in the process of being transported that contained *M. enterolobii* (EPPO Global Database), providing proof that *M. enterolobii* can disperse on plants to further distances. *M. enterolobii* was also found in greenhouses in France (Blok et al., 2002) and Switzerland (Kiewnick et al., 2010) in which suspected *M. enterolobii* infected plants were transported. Therefore, it is vital to know the location of this RKN species because it can spread to further and previously unaffected areas on soil and plants originating from sources in which *M. enterolobii* is present. Knowing the locations in which *M. enterolobii* is located in North Carolina, conducted in this survey, will help to reduce contaminations from field to field, and also help reduce the possible spread of *M. enterolobii* to further distances on infected planting material.

These findings are critical in managing and controlling *M. enterolobii,* especially since management options such as resistant cultivars and non-host crop rotations are limited for *M. enterolobii*. Management practices can be dependent upon the particular species of a pathogen

and are often influenced by the host range of the species (Silva et al., 2018). Crop rotation can be an important tool for reducing nematode damages (Meadows et al., 2018). However, it is vital to know the species of nematode in order to effectively implement a crop rotation plan, as not every species of nematode can infect the same host plants or has the same non-hosts (Sasser and Carter, 1985). For example, a crop rotation study found that rotating from strawberry to corn reduced *M. incognita* and *M. hapla* populations in the soil, however, *Pratylenchus coffeae* and *P. penetrans* were able to infect corn (Chen and Tsay, 2006). Improper or unknown identification of a pathogen species can have implications that reduce the yield and marketability of the crop species (Sasser and Carter, 1985). Therefore, knowing the locations and fields in which *M. enterolobii* is located will help determine an effective crop rotation plan.

In this survey, samples received were correlated to the associated crop in which the sample came from. *M. enterolobii* in this study was detected on tobacco, sweetpotato, and soybean (Table 1). These data provide further insight to tobacco, sweetpotato, and soybean as host plants (Ye et al., 2013; Anonymous, 2014; Gao et al., 2014). Peanut is reported as a non-host to *M. enterolobii* and corn is a suspected poor host (Castagnone-Sereno, 2012; Anonymous, 2014). In this survey, 7 known samples from corn and 17 known samples from peanut did not contain *M. enterolobii*, which provides further insight to the possibility of these two crops as potential rotational crops in fields containing *M. enterolobii*. Only *M. incognita* and *M. arenaria* were identified in samples from peanut and corn, which both of these species have previously been reported to infect corn varieties (Windham and Williams, 1994). However, the absence of *M. enterolobii* in a particular host plant in this survey does not necessarily correlate to the host range of this nematode species. Cotton, wheat, cucurbits, potato, tomato, turf, watermelon, and sage were plants in which samples came from that were not detected to contain *M. enterolobii.*

Host plants previously found to be suitable to infection by *M. enterolobii* include cotton, potato, tomato, and watermelon (Brito et al., 2007; Anonymous, 2014), which were not detected to contain *M. enterolobii* in this study. This could be due to the fact that *M. enterolobii* was simply not present in the particular sample/field. However, research has suggested that plants may be resistant or susceptible to *M. enterolobii* depending on the cultivar of plant (Cervantes-Flores et al., 2002; Chiamolera et al., 2018; Chapter 3).

Host resistance is a management technique that, if available, has had success in controlling nematodes. Chiamolera et al. (2018) found three out of seven guava cultivars were resistant to *M. enterolobii.* A study was conducted to assess differences in tomato cultivars for resistance to major RKN species which found that resistance depended on the cultivar of tomato and species of nematode; The cultivar 'Yoshimatsu' was found to be resistant to *M. javanica* and *M. incognita* race 1, but was susceptible to *M. enterolobii* (Silva et al., 2018). In sweetpotato, the cultivar 'Covington' is resistant to *M. incognita* but is susceptible to *M. enterolobii* (Yencho et al., 2008; Rutter et al., 2019; Chapter 3). Therefore, resistance within crops can depend on the cultivar in addition to the species of nematode. Knowing which nematode species is/are present in a particular area will dictate the management plan, especially for selection of a rotational crop or resistant genotype.

North Carolina is currently under a self-imposed quarantine that restricts movement of sweetpotato planting material infected by *M. enterolobii* (http://www.ncagr.gov/PLANTINDUSTRY/plant/disease/grkn.htm). Knowing where this species of RKN is located helps in limiting movement of infected material and the spread of *M. enterolobii* to new regions (Sasser and Carter, 1985). In addition, knowing where a certain pathogen is located is vital to implement a quarantine; a quarantine is ineffective if the

distribution of the quarantined species is unknown. This survey is an initial study to identify the locations of *M. enterolobii* in North Carolina, and *M. enterolobii* may also be located in more counties than currently identified. Previous surveys of nematode distribution have suggested that increasing the number of samples and increasing the range of geographic distribution of samples typically leads to greater chance of detection of a nematode species (Koenning and Barker, 1998). Increasing the survey geographic distribution can also lead to detection of new species in a particular area, for example, Ye et al. (2015) surveyed RKN species in turfgrass and reported *M. marylandi* in North Carolina and South Carolina for the first time. However, with the importance of knowing the species of RKN to determine a management plan, it is also vital to sweetpotato growers to understand the extent of *M. enterolobii* in order to comply with the quarantine and eliminate the possible spread of this nematode species into previously uninfected fields.

The results from screening the virulence of four North Carolina isolates of *M. enterolobii* on selected resistant and susceptible sweetpotato genotypes found the populations to be homogeneous; no significant differences in infection rates (gall ratings and eggs per gram of root) among the populations were observed. One possible explanation for the four isolates being homogeneous in virulence is that these isolates originated from relatively close geographic proximity in North Carolina (Figure 3). This could mean that more than likely these *M. enterolobii* isolates in North Carolina came from the same source of introduction, or the populations are truly homogeneous. Another possible explanation is that the mitotic parthenogenesis mode of reproduction by *M. enterolobii* has led to homogeneous populations (Koutsovoulos et al., 2019). Despite the difference in isolate origins, these phenotypic results are similar with the genotypic results of Tigano et al. (2010) and Onkendi and Moleleki (2013). Both

studies found that 16 *M. enterolobii* isolates were homogeneous based on DNA analysis (Tigano et al., 2010), and five *M. enterolobii* isolates were homogeneous based on DNA analysis (Onkendi and Moleleki, 2012). However, these results were only based on DNA analysis using IGS and COII amplification products, whereas the study here only compared virulence phenotype and not genotype of *M. enterolobii*. Biotypes can be found among species such as *M. incognita* or *M. arenaria* in which virulence confers to different hosts (Sasser and Carter, 1985; Cervantes-Flores et al., 2002; Dong et al., 2007). Biotypes in RKN species can result in different management methods. For example, having race 3 of *M. javanica* requires planting a pepper variety as a non-host, but having race 3 of *M. javanica* means pepper is a suitable host for this RKN species (Robertson et al., 2009). Cervantes-Flores et al. (2002) found that sweetpotato genotypes had different reactions of resistance based on the species of nematode and even within biotypes of RKN species. Therefore, it is important to continue comparing *M. enterolobii* isolates, especially through differential host tests (Sasser and Carter, 1985), to potentially detect biotypes that confer varying degrees of virulence in order to manage *M. enterolobii* long-term.

The survey for identification of the dispersion of *M. enterolobii* in eastern North Carolina contributes to understanding the at-risk crops and counties which are under threat due to this RKN species and helps to potentially limit the spread of *M. enterolobii* by increasing awareness. The four populations of *M. enterolobii* obtained from North Carolina are considered to be homogeneous based on phenotypic sweetpotato host tests. These results can have implications on efficient management plans in *M. enterolobii* populated fields.

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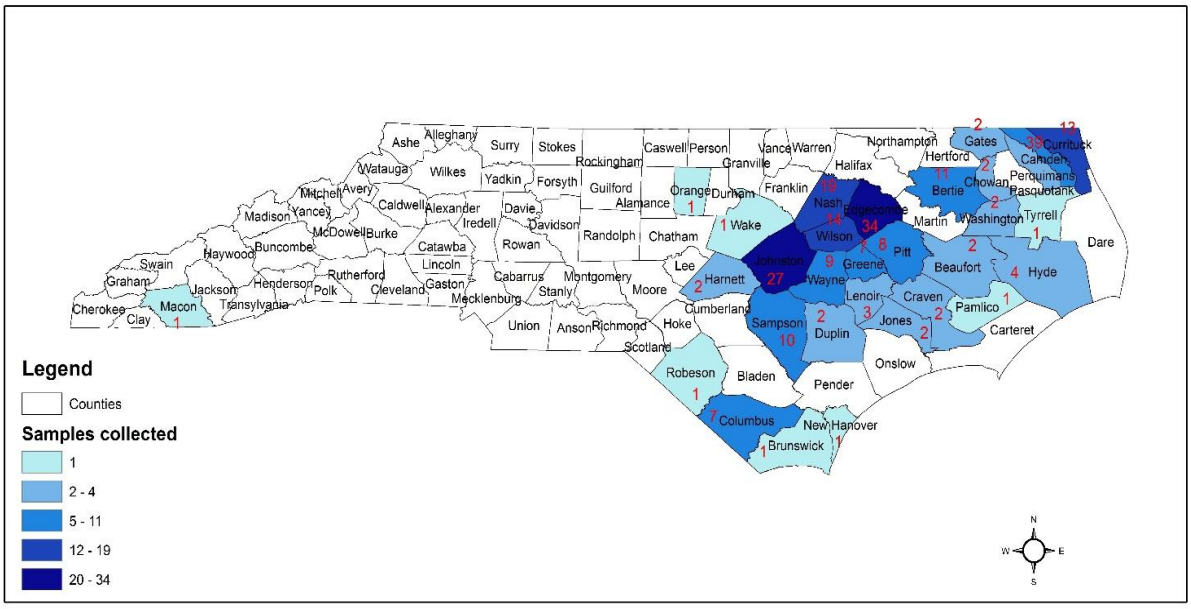

Figure 1. County map of North Carolina in which root-knot nematode positive samples were collected and confirmed to the genus level by the NCDA&CS Nematode Assay Lab. Red numbers represent the number of RKN-positive samples collected from the respective county and analyzed in this survey.

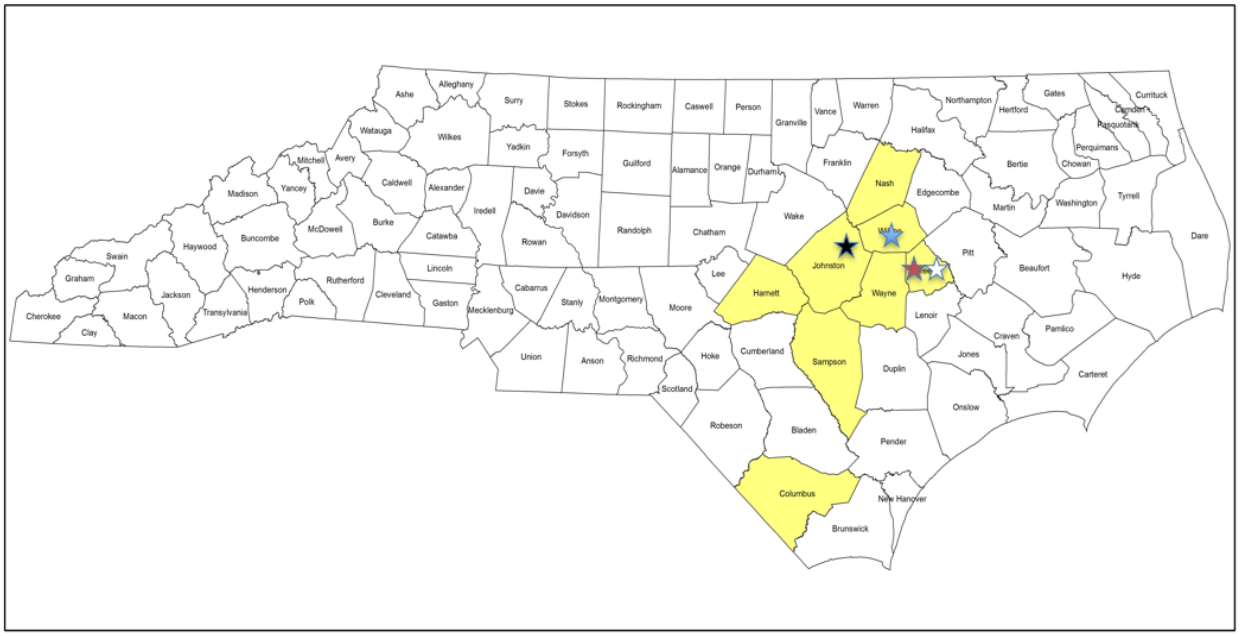

Figure 2. Counties that are highlighted in yellow are confirmed counties containing *Meloidogyne enterolobii.* Stars represent the approximate location of where each of the four sources of *M. enterolobii* populations used to start cultures originated from. Red star- ‘ent11’ population from infected sweetpotato storage root in Greene County. Blue star- ’18-6908’ population from infected sweetpotato storage root in Wilson County. White star- ’18-8260’ population from infected sweetpotato storage root in Greene County. Black star- ’18-5126’ population from infected soil of soybean field in Johnston County.

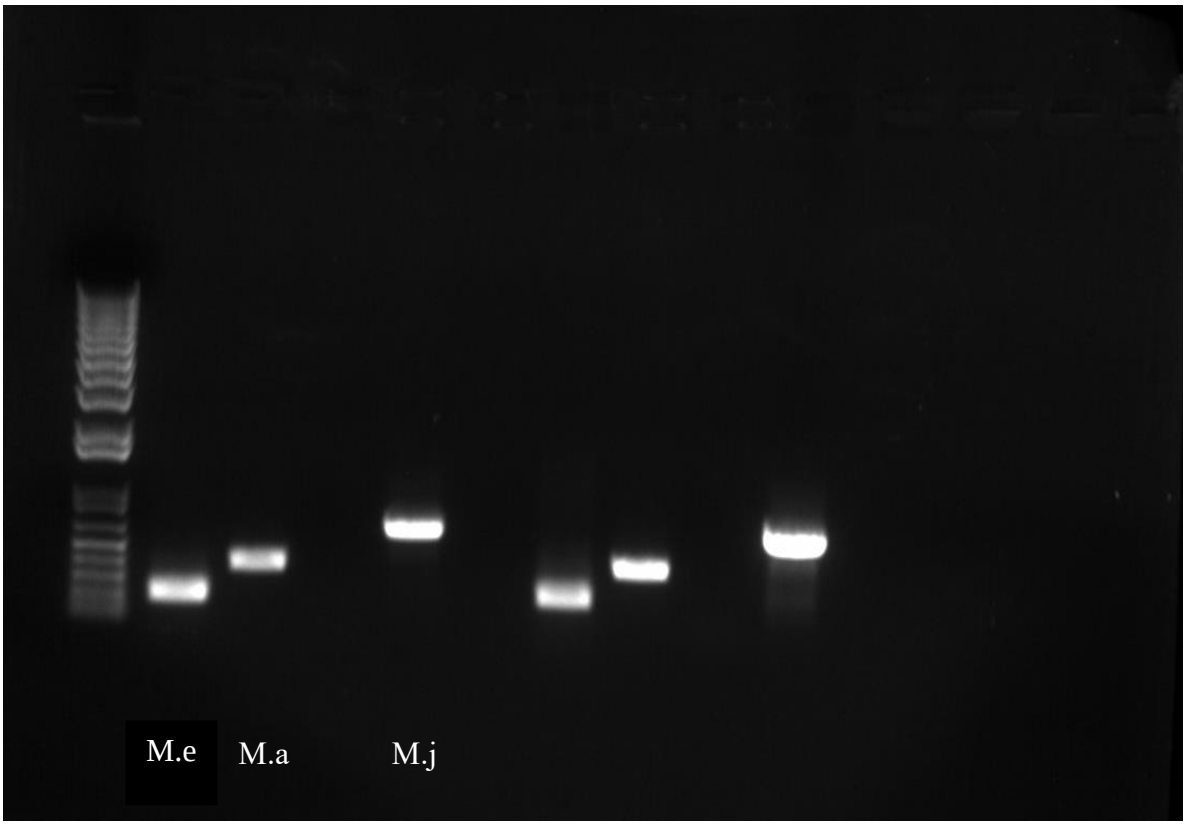

Figure 3. Results from gel electrophoresis. Known samples of DNA from *M. enterolobii, M. arenaria,* and *M. javanica* (labeled on gel) were run to compare to unknown RKN-species samples. Results show the unknown samples on the right-half of the gel match the known samples on the left half of the gel.

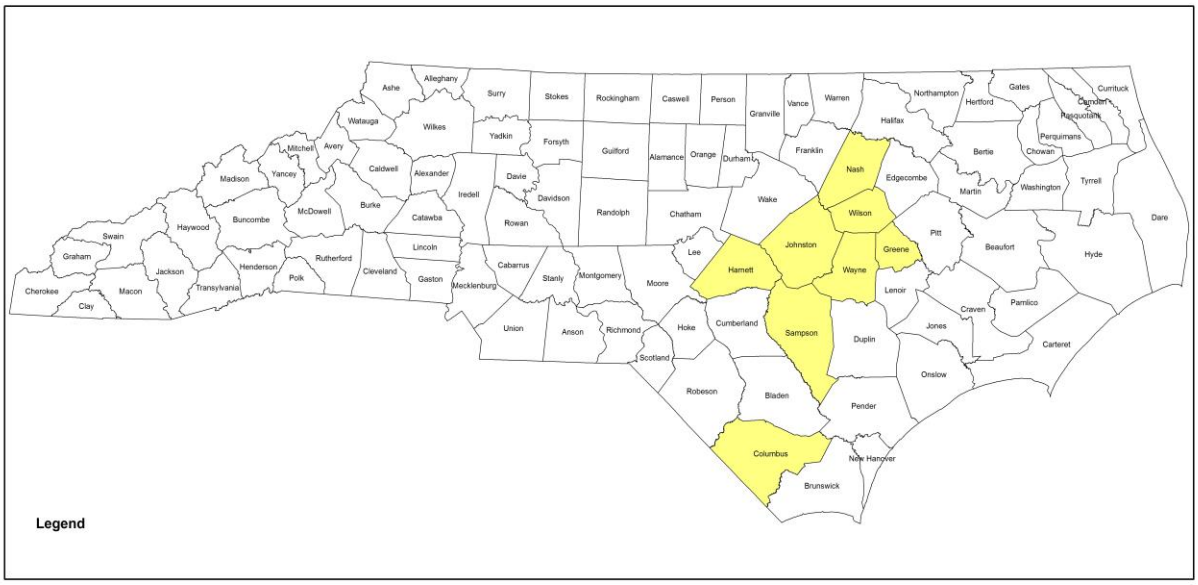

Figure 4. North Carolina counties (highlighted) confirmed to have *Meloidogyne enterolobii* after completion of the survey reported here.

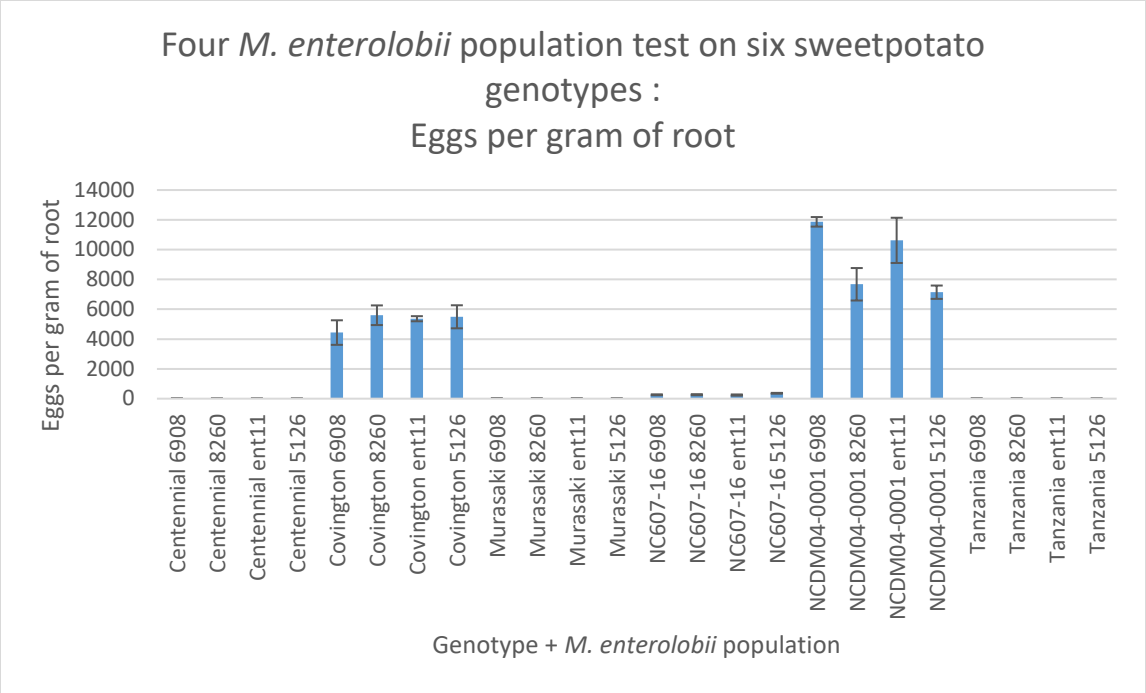

Figure 5. The number of eggs per gram of root produced by four independent *Meloidogyne enterolobii* isolates from North Carolina: ’18-6908’, ’18-8260’, ‘ent11’, ’18-5126’ on six selected sweetpotato genotypes. Sweetpotato genotypes: ‘Centennial’, ‘Covington’, ‘Murasaki-29’, ‘NC607-16’, ‘NCDM04-0001’, ‘Tanzania’. Table values represent the mean of four isolates. Fisher’s *t* test (alpha=.05) using data transformed log (x+1) found sweetpotato genotype to be significant ($P < 0.001$) and *M. enterolobii* population was not significant.

Table 1. List of eastern North Carolina crops with RKN-positive samples that were used for *Meloidogyne* species identification in this investigation. Crops associated with *M. enterolobii*-positive samples shown.

Crop	Number of samples tested	Number of *M. enterolobii*-positive samples (% of samples from 12 total *M. enterolobii* samples)
Tobacco	34	4 (33.33%)
Sweetpotato	28	4 (33.33%)
Soybean	25	2 (16.66%)
Peanut	17	0
Cotton	9	0
Wheat	9	0
Corn	7	0
Cucurbits	4	0
Potato	4	0
Tomato	2	0
Turf	1	0
Watermelon	1	0
Sage	1	0
Unknown crop	61	2 (16.66%)

Table 2. PCR primers that amplify DNA products that specifically identify the *Meloidogyne* species *M. enterolobii, M. javanica, M. arenaria, M. incognita,* and *M. hapla.*

Primer and target species	Amplified gene	Direction	Sequence	Reference
MeF (*M. enterolobii*)	IGS2	F	AACTTTTGTGAAAGTGCCGCTG	Long et al., 2006
MeR (*M. enterolobii*)	IGS2	R	TCAGTTCAGGCAGGATCAACC	Long et al., 2006
MK7 (*M. enterolobii*)	SCAR	F	GATCAGAGGCGGGCGCATTGCGA	Tigano et al, 2010
MK7 (*M. enterolobii*)	SCAR	R	CGAACTCGCTCGAACTCGAC	Tigano et al, 2010
Fjav (*M. javanica*)	SCAR	F	GGTGCGCGATTGAACTGAGC	Zijlstra et al., 2000
Rjav (*M. javanica*)	SCAR	R	CAGGCCCTTCAGTGGAACTATAC	Zijlstra et al., 2000
Far (*M. arenaria*)	SCAR	F	TCGGCGATAGAGGTAAATGAC	Zijlstra et al., 2000
Rar (*M. arenaria*)	SCAR	R	TCGGCGATAGACACTACAACT	Zijlstra et al., 2000
Finc (*M. incognita*)	SCAR	F	GGTGCGCGATTGAACTGAGC	Zijlstra et al., 2000
Rinc (*M. incognita*)	SCAR	R	CTCTGCCCTCACATTAAG	Zijlstra et al., 2000

Table 2 (Continued).

M. h. (JMV)(*M. hapla)*	SCAR	F	TTTCCCCTTATGATGTTTACCC	Wishart et al., 2002
M.h. (JMV)(*M. hapla*)	SCAR	R	AAAAATCCCCTCGAAAAATCCACC	Wishart et al., 2002

Resistance of Sweetpotato Genotypes to *Meloidogyne enterolobii*

Abstract. Potential resistance to *Meloidogyne enterolobii* in ninety-one selected sweetpotato [*Ipomoea batatas* (L.) Lam.] genotypes was evaluated in five greenhouse experiments. Ten thousand eggs of *M. enterolobii* were inoculated on each sweetpotato genotype grown in a 3:1 sand to soil mixture. Sixty days post inoculation, roots were weighed, galls were rated based on percent of total roots with galls, and nematode eggs were extracted from roots. Genotype susceptibility to *M. enterolobii* was assessed as the number of nematode eggs per gram of root. Based on the statistical means separation by Fisher's LSD *t* test, the threshold for differentiating susceptible and resistant genotypes was set as 500 eggs per gram of root. Differences in sweetpotato genotypes were found, as genotypes were significant ($P < 0.001$) in all five tests for gall rating, total eggs, and eggs per gram of root. Results concluded that 20 out of 91 tested sweetpotato genotypes were resistant to *M. enterolobii*. Some of the susceptible genotypes included 'Covington', 'Beauregard', 'NCDM04-001', and 'Hernandez'. Some of the resistant sweetpotato genotypes included 'Tanzania', 'Murasaki-29', 'Bwanjule', 'Dimbuka-Bukulula', 'Jewel', and 'Centennial'. Most of the 20 resistant sweetpotato genotypes supported less than 20 eggs/g root of *M. enterolobii.* A number of segregants from a 'Tanzania' x 'Beauregard' cross demonstrated the strong resistance to *M. enterolobii* observed in the 'Tanzania' parent. In collaboration with NC State University sweetpotato breeders, several of the genotypes evaluated in the above tests are now being used to incorporate the observed resistance to *M. enterolobii* into commercial sweetpotato cultivars.

Introduction

Of all the plant-parasitic nematodes, root-knot nematodes (RKN, *Meloidogyne* spp.) cause significant damage to economically valuable crops (Sasser and Carter, 1985; Jones et al., 2013). There are approximately 100 described species of *Meloidogyne* (Mitkowski and Abawi, 2003). They parasitize a wide range of hosts, with more than 2,000 plant species known to be suitable to infection (Sasser and Freckman, 1987). Among the host range, RKN can pose a serious threat to sweetpotato production (Lawrence et al., 1986; Anonymous, 2014). RKN infection, feeding, and galling of sweetpotato plant roots causes reductions in plant vigor and yield (Lawrence et al., 1986; Overstreet, 2013). In addition, RKN also infect the sweetpotato storage roots causing characteristic galls and cracks that can further reduce the value, storage, and marketability of harvested sweetpotatoes (Lawrence et al., 1986; Cervantes-Flores et al., 2002). Proper nematode management often involves a single or combination of methods such as nematode-free planting material, crop rotation to non-hosts, nematicides, and resistant cultivars (Sasser and Carter, 1985; Lawrence et al., 1986; Lambert and Bekal, 2002). An effective, cost-efficient, and environmentally suitable method is through host genotype resistance (Clark and Moyer, 1988; Villordon and Clark, 2018).

Previous research on sweetpotato genotype resistance to RKN has been conducted. A study in 1986 involved testing resistance of the sweetpotato genotypes ‘Centennial’ and ‘Jasper’ to *M. incognita* which resulted in both being susceptible (Lawrence et al., 1986). An extensive study of sweetpotato genotype resistance included the RKN species *M. incognita*, *M. arenaria*, and *M. javanica* (Cervantes-Flores et al., 2002). Cervantes-Flores et al. (2002) were able to identify a few sweetpotato genotypes that demonstrated resistance to one, if not all, of the three

RKN species studied. However, no extensive study has been reported on sweetpotato genotype resistance to the newly emerging RKN *M. enterolobii* (Castagnone-Sereno, 2012).

Meloidogyne enterolobii (the guava root-knot nematode) is an aggressive pathogen that currently has, to our knowledge, a limited distribution in the United States. *M. enterolobii* was first described in China in 1983 (Yang and Eisenback, 1983). It has since been detected in countries in Africa, Asia, South America, and North America (Anonymous, 2014; Brito et al., 2007). In the United States, this species has been reported in Florida in 2004 (Brito et al., 2004), North Carolina in 2013 (Ye et al., 2013), Louisiana in 2019 (Hare, 2019) and South Carolina in 2019 (Rutter et al., 2019). *M. enterolobii* has been found to infect and reproduce on hosts with RKN resistance genes such as *Mi-1* in tomato and *N*-gene peppers (Kiewnick et al., 2009; Castagnone-Sereno, 2012). *M. enterolobii* has also been reported infecting the *M. incognita* resistant sweetpotato cultivar 'Covington' (Rutter et al., 2019). If a host plant is susceptible to *M. enterolobii*, the nematodes parasitize and establish inside the roots where reproduction occurs (Sasser and Carter, 1985; Lambert and Bekal, 2002). However, in a resistant genotype, the nematodes are inhibited in reproduction, and populations decline as a result (Sasser and Carter, 1985; Lambert and Bekal, 2002).

In the United States, North Carolina is the nation's largest sweetpotato producing state (USDA, 2018). North Carolina harvested an estimate of 78,500 acres of sweetpotatoes, or $236,285,000 value of production in 2018 (USDA, 2018). This value represents more than half of the sweetpotato production in the United States (Barkley et al., 2017). However, sweetpotato production in North Carolina is under threat due to *M. enterolobii* emerging as a nematode threat in the state (Ye et al., 2013). In North Carolina, the sweetpotato cultivar 'Covington' was granted a plant patent in 2008 and has been the prominent cultivar grown by North Carolina farmers

since its introduction (Yencho et al., 2008). ‘Covington’ accounts for 88% of the acreage in North Carolina (Barkley et al., 2017). In addition, ‘Beauregard’ is also a prominent cultivar grown in sweetpotato producing states (Cervantes-Flores et al., 2002). ‘Beauregard’ has been shown to be resistant to the RKN *M. arenaria* race 2, but susceptible to *M. javanica* and *M. incognita* (Cervantes-Flores et al., 2002). ‘Covington’ had been a successful cultivar due to superior agronomic quality, such as taste and ability to grow in this region, and its high disease resistance, including resistance to *M. incognita* (Yencho et al., 2008). The ability of *M. enterolobii* to infect ‘Covington’ sweetpotato (Rutter et al., 2019) makes screening sweetpotato genotypes for resistance to *M. enterolobii* critical. There is currently a lack of known resistance genes in sweetpotato that confer resistance to *M. enterolobii.* In collaboration with the sweetpotato breeding program at North Carolina State University, selected sweetpotato genotypes were screened in this study in replicated greenhouse tests for potential resistance to *M. enterolobii* in order to identify resistant genotypes.

Materials and Methods

Sweetpotato genotypes were evaluated for potential resistance to *Meloidogyne enterolobii* in replicated greenhouse trials. Ninety-one sweetpotato genotypes were evaluated over a series of five separate trials. Each genotype was replicated five times in a single trial and arranged in a completely randomized design. Sweetpotato genotypes were chosen based on previous results of RKN screening of species other than *M. enterolobii,* the genetic origin of the genotype, and availability of germplasm from the breeding program at North Carolina State University. ‘Tanzania’ was chosen as a check sweetpotato genotype in trials because of previously reported resistance to several RKN species (Cervantes-Flores et al., 2002).

Sweetpotato cultivars used as checks included ‘Beauregard’ which is susceptible to *M. incognita* (Karuri et al., 2016), ‘Covington’ which is resistant to *M. incognita* (Yencho et al., 2008), ‘Hernandez’ which is moderately resistant to *M. incognita* (Cervantes-Flores et al., 2002), and ‘Ruddy’ which is resistant to *M. incognita* (Bohac et al., 2002). Test genotypes found to be susceptible to *M. enterolobii* in one trial were not re-tested since five replications were considered sufficient to assess the cultivar as not resistant. Genotypes that displayed resistance to *M. enterolobii* were re-tested for further confirmation.

Twelve to fifteen-cm-long sweetpotato cuttings (slips) were planted in 4-inch deep plastic pots containing a 3:1 sand soil mixture (88.9% sand, 8.2% silt, 2.8% clay) in the greenhouse. A population of *M. enterolobii* that was isolated from a soybean field rotated with sweetpotato in Johnston County, North Carolina, was cultured on ‘Rutgers’ tomato plants (*Lycopersicon lycopersicum*) in the greenhouse. Fourteen days after planting the cuttings to allow rooting, 10,000 eggs of *M. enterolobii* were introduced about 2-cm deep into the soil surrounding the roots of each sweetpotato plant. Plants were grown in a greenhouse under conditions of about 25°C to 28°C and watered once per day.

Plants were evaluated at 60 days post inoculation with *M. enterolobii*. The plants were cut at the crown to isolate the roots from top growth. Each root was rinsed of soil and the fresh weight of each root system was recorded. Visual gall ratings were done based on the total percent of the root system galled. RKN eggs were then extracted from each whole root system using the NaOCl method described by Hussey and Barker (1973) and recovered on a 25µm-opening sieve.

Aliquots of eggs from each root system were counted using an inverted microscope. The total egg count was divided by the fresh weight of the root to provide the number of eggs per

gram of root. The host suitability of sweetpotato genotype to *M. enterolobii* was assessed through the number of eggs per gram of root.

Data was analyzed using SAS statistical analysis software (SAS Institute, Cary, NC). Each trial was analyzed independently as trials were conducted at separate times, and data values were averaged across replications within each trial. Data was analyzed in SAS as a general linearized model (PROC GLM). All data were transformed by log(x+1) to standardize variance for statistical analyses. Transformed means were separated using Fisher's *t* test LSD procedure (alpha=0.05). Values presented in tables (Tables 1-5) represent the mean of non-transformed data. Based upon Fisher's *t* test performed to separate the means, sweetpotato genotypes that supported 500 eggs/g root or less were classified as resistant to *M. enterolobii.*

Results

There were significant differences among sweetpotato genotypes ($P < 0.001$) for gall rating, total eggs, and eggs per gram of root. Gall ratings among sweetpotato genotypes were significantly different ($P < 0.001$); all resistant cultivars had gall ratings of 0% with the exception of genotype 'CN1058-3' which had a mean gall rating of 3%. Genotypes were classified as resistant if it supported 500 or less eggs/g root.

Among the 20 different genotypes screened against *M. enterolobii* in Test 1, 'Tanzania' (2.3 eggs/g root) was the only resistant cultivar with low egg production by *M. enterolobii* (Table 1). Among the susceptible genotypes in Test 1, 'Ruddy' (2,952 eggs/g root) had the least amount of eggs per gram of root but was significantly greater compared to 'Tanzania'. 'Naspot 8' (11,009 eggs/g root) was the most susceptible genotype in Test 1 as it had the most eggs per

gram of root. Besides ‘Tanzania’, the other check sweetpotato genotypes in Test 1 were all susceptible to *M. enterolobii* (Table 1).

In the 22 total genotypes analyzed in Test 2, five were identified as resistant to *M. enterolobii*: ‘Tanzania’ (1.8 eggs/g root) was again identified as resistant, and ‘Jewel’ (1.9 eggs/g root), ‘Murasaki-29’ (3.0 eggs/g root), ‘Bwanjule’ (5.1 eggs/g root), and ‘Dimbuka-Bukulua’ (11.3 eggs/g root) displayed resistance to *M. enterolobii* (Table 2). The 0% gall ratings were consistent among resistant genotypes. All checks screened in Test 2 were consistent in terms of susceptibility with the results from Test 1 (Table 2), as ($P < 0.001$) was significant for eggs per gram of root and gall ratings among checks.

Test 3 contained 25 total genotypes in which four were resistant to *M. enterolobii* (Table 3). ‘Tanzania’ (1.5 eggs/g root) was confirmed again as resistant, and ‘Centennial’ (2.9 eggs/g root), ‘Tib 11’ (10.8 eggs/g root), and ‘NC607-16’ (437.9 eggs/g root) were also classified as resistant. Genotype ‘NC607-16’ had several hundred more eggs per gram of root compared to the other resistant cultivars; however, the genotype had less than 500 eggs per gram of root and a mean gall rating of 0% (Table 3). When comparing susceptible genotypes to resistant, ‘NC607-16’ (437.9 eggs/gram root) had significantly fewer eggs per gram of root than the closest susceptible genotype ‘NC08-0553’ (2,344 eggs/gram root) (Table 3). All checks in Test 3 were consistent with Tests 1 and 2 in terms of susceptibility in correlation with eggs/g root ($P < 0.001$).

Test 4 had six *M. enterolobii* resistant genotypes out of a total of 24 genotypes screened (Table 4). Resistant genotypes included ‘L50’ (4.1 eggs/g root), ‘Mojave’ (4.5 eggs/g root), ‘Red Resisto’ (7.4 eggs/g root), ‘Tanzania’ (9.3 eggs/g root), ‘Resisto’ (17.2 eggs/g root), and ‘CN1058-3’ (482.3 eggs/g root). ‘CN1058-3’ was another genotype considered having potential

quantitative resistance that had less than 500 eggs per gram of root. While a low percentage of galls were found on the roots of 'CN1058-3', galls did not necessarily correlate to egg production. The response of checks included in this test (Table 4) to *M. enterolobii* were consistent with previous tests in terms of susceptibility and eggs per gram of root.

Test 5 yielded 14 out of 23 genotypes as resistant to *M. enterolobii* (Table 5). A number of sweetpotato genotypes found to be resistant to *M. enterolobii* in above tests were re-tested in Test 5. New genotypes in this test that were found to be resistant were 'TB-019' (6.5 eggs/g root), 'TB-068' (7.6 eggs/g root), 'Wagabolige' (7.4 eggs/g root), 'TB-056' (8.6 eggs/g root), 'TB-146' (9.5 eggs/g root), 'TB-257' (10.4 eggs/g root), and 'Pelican Processor' (14.6 eggs/g root). Genotypes that were resistant in Test 5 that were also included in previous tests were 'Tanzania' (3.4 eggs/g root), 'Jewel' (4.9 eggs/g root), 'Centennial' (7.4 eggs/g root), 'Tib 11' (8.6 eggs/g root), 'Dimbuka-Bukulula' (10.5 eggs/g root), 'Bwanjule' (11.3 eggs/g root), and 'Murasaki-29' (11.4 eggs/g root). These results are consistent with previous tests in terms of susceptibility of genotypes. All resistant sweetpotatoes in Test 5 had a mean gall rating of 0%. As in all previous tests, eggs per gram of root between all resistant and susceptible genotypes was significantly different ($P < 0.001$) (Table 5).

Discussion

Use of resistant genotypes can be effective in managing nematodes (Lawrence et al., 1986; Trudgill, 1991). Throughout five tests in this study, 20 out of 91 sweetpotato genotypes screened were identified as resistant to *M. enterolobii*. Previous studies have identified resistant sweetpotato genotypes to other RKN species such as *M. incognita* (Jatala and Russell, 1972; Cervantes-Flores et al., 2002; Karuri et al., 2017). A study by Rutter et al. (2019) identified that

the sweetpotato genotype ‘Covington’ was susceptible to *M. enterolobii*. However, to our knowledge this is the first report of multiple sweetpotato genotypes screened with resistance to *M. enterolobii.*

The identification of resistant sweetpotato genotypes to *M. enterolobii* is important to sweetpotato production, especially where this species of RKN is located. Countries such as China, Vietnam, the United States, and many countries in Africa are important sweetpotato producers (Tan, 2015) that also harbor *M. enterolobii* (Anonymous, 2014; Gao et al., 2014; Subbotin, 2019). Locations that grow sweetpotatoes that are under threat due to *M. enterolobii* can utilize these results to potentially incorporate a resistant genotype in their field that is cost effective and does not require the use of nematicides (Lambert and Bekal, 2002). In less developed countries, resistant genotypes may be the only economically viable form of management (Roberts, 1992).

The sweetpotato genotypes screened in this study are diverse in disease resistance, optimal climate for growth, sweetpotato taste and color, and geographic origins (La Bonte et al., 2008; Yencho et al., 2008; Ddumba et al., 2014). Not every sweetpotato genotype is acclimated to grow in every region (Ddumba et al., 2014), therefore, selection of sweetpotato genotype is critical especially if the field is under pressure from *M. enterolobii.*

There were numerous sweetpotato genotypes from African origins that were included in this study. ‘Tanzania’ was found to be resistant to *M. enterolobii* and is a commonly grown genotype in Africa (Ddumba et al., 2014). ‘Tanzania’ is an African landrace that was found to be resistant to *M. incognita* (Cervantes-Flores et al., 2002; Karuri et al., 2016), and also has resistance to other diseases such as sweetpotato virus disease (SPVD) (Ddumba et al., 2014). ‘Tanzania’ represents a widely grown sweetpotato in Africa (Ddumba et al., 2014), and results

from this study suggest that ‘Tanzania’ can be a genotype option to grow where *M. enterolobii* is found in Africa. The genotype ‘Dimbuka-Bukulula’ was resistant to *M. enterolobii*, which also has other disease resistance such as Alternaria stem blight (Ddumba et al., 2014). ‘Bwanjule’ is a genotype with resistance to *M. enterolobii* that also has resistance to sweetpotato weevils (Ddumba et al., 2014). ‘Wagabolige’ also represents an African genotype with resistance to *M. enterolobii* that also is resistant to *M. incognita* and SPVD (Cervantes-Flores et al., 2002; Ddumba et al., 2014). These sweetpotato genotypes, which are all resistant to *M. enterolobii*, also have a relatively sweet taste that is desired by most sweetpotato consumers (Ddumba et al., 2014). ‘Tanzania’, ‘Bwanjule’, ‘Dimbuka-Bukulula’, and ‘Wagabolige’ are rated as having a moderately sweet taste (Ddumba et al., 2014). Although these genotypes do not grow well in the United States (Craig Yencho, personal communication), these genotypes are options that growers can utilize in *M. enterolobii* infested fields as a management strategy, especially in Africa, that have other horticultural qualities such as Alternaria stem blight resistance and a relatively desirable taste that convey resistance to *M. enterolobii.*

In addition, a few sweetpotato genotypes originating from the United States were classified as resistant to *M. enterolobii.* ‘Murasaki-29’ is a genotype released by the Louisiana Agricultural Experiment Station that has certain desirable horticultural characteristics (Arnold, 2016). It is a purple skin sweetpotato with resistance to Fusarium wilt, Rhizopus soft rot, Fusarium root rot, and to the RKN species *M. incognita* (Arnold, 2016). ‘Jewel’, released by North Carolina Agricultural Research Service, is resistant to *M. incognita*, and has intermediate resistance to Fusarium wilt, Rhizopus soft rot, and bacterial root rot (Arnold, 2016). ‘Pelican Processor’ was found to be resistant to *M. arenaria* (Cervantes-Flores, 2000), which is also resistant to *M. enterolobii*. ‘Resisto’, developed in South Carolina, is rated as having a highly

desirable taste and good storage quality (Kuepper and Freeman, 2013), which is also resistant to *M. enterolobii.* In addition, resistance seems to have successfully conferred from a parent genotype; 'Centennial' is the parent to the sweetpotato genotype 'Jewel' (Craig Yencho, personal communication), in which both of these genotypes were resistant to *M. enterolobii* in this study. These genotypes represent possible options that growers can choose that not only have resistance to other pathogens, but also with resistance to *M. enterolobii.* These genotypes are especially critical in the United States, as they have been marketable options in the past (Arnold, 2016).

The varying quantity of eggs per gram of root among resistant genotypes suggests that quantitative and qualitative resistance could be present among the resistant sweetpotato genotypes (Jones and Dukes, 1980; Cervantes-Flores et al., 2008). Qualitative resistance may be active in resistant lines that supported less than 20 eggs per gram of root, as egg production was almost completely inhibited. Quantitative resistance may be found in the sweetpotato genotypes that supported between 400-500 eggs per gram of root, such as 'NC607-16' and 'CN1058-3'. Studies have found that resistance to RKN may be conferred by multiple genes (Cervantes-Flores et al., 2002), and resistance in genotypes may have multiple origins (Mcharo et al., 2005; Vasudevan et al., 2016). Therefore, results suggest that there may be more than one gene conferring resistance to *M. enterolobii* among the sweetpotato genotypes tested.

The sweetpotato check genotypes produced interesting results as only 'Tanzania' and 'Beauregard' were consistent with previous data based on screening against the major RKN species *Meloidogyne incognita*, as 'Tanzania' was resistant to *M. enterolobii* and *M. incognita* and 'Beauregard' was susceptible to *M. enterolobii and M. incognita* (Cervantes-Flores et al., 2002; Karuri et al, 2016). However, the genotypes 'Covington', 'Hernandez', and 'Ruddy' were

susceptible to *M. enterolobii* despite having resistance to *M. incognita* (Cervantes-Flores et al., 2002).

In North Carolina, ‘Covington’ is the most widely grown sweetpotato due to many favorable horticultural characteristics including resistance to *M. incognita* (Yencho et al., 2008; Barkley et al., 2017). Since ‘Covington’ represents about 88% of sweetpotatoes grown in North Carolina (Barkley et al., 2017), the sweetpotato industry, especially in North Carolina, is under pressure due to the susceptibility of ‘Covington’ to *M. enterolobii*. Data on sweetpotato genotypes with resistance to *M. enterolobii* is limited, however, Rutter et al. (2018) also found ‘Covington’ to be susceptible to *M. enterolobii.*

In addition, ‘Beauregard’ is a popular variety in the southeastern United States (Overstreet, 2013). Results from these tests show that ‘Beauregard’ is also susceptible to infection by *M. enterolobii*. However, ‘Beauregard’ has great qualities that many breeding programs desire, but the threat of RKN may prevent this genotype from being commonly grown. ‘Tanzania’ sweetpotato was found to have resistance to several RKN species in previous tests (Cervantes-Flores et al., 2002). The North Carolina State University sweetpotato breeding program has produced a mapping population that resulted from crossing ‘Beauregard’ and ‘Tanzania’ (Craig Yencho, personal communication). Selections from the ‘TB’ mapping population were introduced in Test 5 here to screen for potential resistance to *M. enterolobii*. Eight total ‘TB’ genotypes were included in Test 5, with six out of the eight genotypes being resistant to *M. enterolobii,* suggesting that the mapping population is segregating for broad RKN resistance. The results of crossing ‘Beauregard’ and ‘Tanzania’ will yield a more conclusive insight into the gene or genes that confer resistance to *M. enterolobii*.

The results from this study will lead to improved management of *M. enterolobii*, as sources of sweetpotato resistance to *M. enterolobii* have been identified. Genetic host resistance is cost efficient and an environmentally friendly method to control nematodes (Sasser and Carter, 1985; Lambert and Bekal, 2002). To our knowledge, this is the first report of different sweetpotato genotype resistance to *M. enterolobii*. Results from Lawrence et al. (1986), and the success of *M. incognita* resistant 'Covington' from greenhouse to field (Yencho et al., 2008), suggests that the resistance to *M. enterolobii* will be transferable to field conditions. The discovery of resistant sweetpotato genotypes to *M. enterolobii* represents a vital management tool in locations where this RKN species is located.

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Table 1. Galling and reproduction (eggs) of *Meloidogyne enterolobii* observed in roots of selected sweetpotato genotypes in greenhouse Test 1.

Genotype	Gall rating[a]	Eggs/root system	Eggs/gram root	Susceptible/Resistant[b]
Tanzania	0 a	124 a	2.3 a	R
Ruddy	1.8 b	124,459 b	2,952 b	S
Hernandez	4.4 c	202,333 bcde	3,268 bc	S
Bonita	6.2 cde	168,333 bcd	2,918 bcd	S
NC04-0531	1.4 b	172,333 bc	4,193 bcd	S
Naspot 4	11.2 gh	242,166 cdef	3,819 bcde	S
Covington	5.2 c	200,333 bcde	3,748 bcde	S
Averre	8 efg	251,333 bcdef	4,676 bcde	S
New Kawogo	4.4 cd	280,499 defg	4,236 bcdef	S
Beauregard	8.6 defg	281,333 cdef	4,865 bcdef	S
Naspot 2	8 efg	327,666 defg	4,773 bcdef	S
Bayou Belle	10.4 fgh	256,333 bcdef	5,248 bcdef	S
Bellevue	5.8 cde	227,999 bcdef	4,975 bcdef	S
Naspot 5	6.2 cdef	230,333 bcdef	5,107 bcdef	S
Kakamega	4.4 cd	236,499 bcdef	5,492 cdefg	S
Naspot 10	8 defg	378,333 fg	5,874 defg	S
Naspot 3	9.2 efgh	314,833 efg	5,932 efg	S
Naspot 9	6.6 cde	445,833 fgh	7,490 efg	S
Naspot 1	15.4 h	501,550 gh	7,809 fg	S
Naspot 8	10.8 gh	709,166 h	11,009 g	S

All table values are the mean of five replications of sweetpotato plant roots inoculated with 10,000 eggs of *M. enterolobii* and scored at 60 days after inoculation. Column values followed by the same letter are not significantly different according to Fisher's *t* test (alpha = .05) using data transformed log (x+1).

[a] Gall ratings represent visual quantification of percent of total root system with galls.

[b] Resistant (R) = sweetpotato genotypes that supported less than 500 eggs/gram root. Susceptible (S) = sweetpotato genotypes that supported more than 500 eggs/gram root.

Table 2. Galling and reproduction (eggs) of *Meloidogyne enterolobii* observed in roots of selected sweetpotato genotypes in greenhouse Test 2.

Genotype	Gall rating[a]	Eggs/root system	Eggs/gram root	Susceptible/Resistant[b]
Jewel	0 a	88 a	1.9 a	R
Tanzania	0 a	79 ab	1.8 ab	R
Murasaki-29	0 a	103 ab	3.0 abc	R
Dimbuka-Bukulua	0 a	156 b	11.3 bc	R
Bwanjule	0 a	133 b	5.1 c	R
Kokie-14	3.0 bc	132,833 c	2,593 d	S
NCP13-0285	3.0 b	147,666 cd	2,859 d	S
Evangeline	4.8 cdef	148,000 cde	3,750 de	S
NCP13-0315	2.4 b	203,330 cdef	3,779 de	S
MC14-0363	5.6 efg	221,833 cdef	4,472 de	S
Beauregard	4.8 cdef	258,541 defg	4,067 de	S
NCP06-0020	3.8 bcde	253,500 cdef	5,270 def	S
NC11-0234	6.4 fgh	330,246 fg	6,174 efg	S
NC07-0847	5.0 def	444,833 ghi	6,612 efgh	S
NCP13-0005	3.4 bcd	271,500 efg	7,154 efgh	S
NCP13-0300	5.4 ef	615,833 hi	9,573 fgh	S
FTA-94	5.2 ef	509,666 ghi	9,986 fgh	S
NC413	8.8 hi	339,333 fgh	11,123 gh	S
NC13-1027	11.2 i	432,000 ghi	11,584 gh	S
NC09-0122	7.8 ghi	403,166 fghi	12,443 hi	S
Burgundy	9.0 i	339,500 fgh	12,611 hi	S
DM02-0180	10.2 i	666,033 i	22,208 i	S

All table values are the mean of five replications of sweetpotato plant roots inoculated with 10,000 eggs of *M. enterolobii* and scored at 60 days after inoculation. Column values followed by the same letter are not significantly different according to Fisher's *t* test (alpha = .05) using data transformed log (x+1).

[a] Gall ratings represent visual quantification of percent of total root system with galls.

[b] Resistant (R) = sweetpotato genotypes that supported less than 500 eggs/gram root. Susceptible (S) = sweetpotato genotypes that supported more than 500 eggs/gram root.

Table 3. Galling and reproduction (eggs) of *Meloidogyne enterolobii* observed in roots of selected sweetpotato genotypes in greenhouse Test 3.

Genotype	Gall rating[a]	Eggs/root system	Eggs/gram root	Susceptible/Resistant[b]
Tanzania	0 a	58 a	1.5 a	R
Centennial	0 a	158 b	2.9 a	R
Tib 11	0 a	326 b	10.8 b	R
NC607-16	0 a	7,963 c	437.9 c	R
NC08-0553	0.8 b	92,666 d	2,344 d	S
Norin-2	3.4 cd	176,333 defg	3,705 de	S
Covington	5.3 ef	163,333 def	3,713 def	S
Beauregard	4.3 def	291,388 fghij	3,858 def	S
W392	0.6 b	205,500 defgh	3,941 def	S
Hernandez	3.7 de	228,333 efgh	4,905 efg	S
Ruddy	2.0 c	132,500 de	4,931 efg	S
NC11-0805	8.4 gh	278,166 efgh	7,477 fgh	S
NC03-0302	6.2 fg	390,833 hij	7,467 ghi	S
BM85-42	3.2 cd	312,000 ghij	7,170 ghi	S
HiDry	1.0 b	164,833 de	9,925 ghi	S
Excel	8.0 gh	381,500 ghij	9,961 ghi	S
Suwon-127	3.4 cde	357,333 fghij	9,589 ghi	S
NC08-0435	8.8 ghi	234,500 efgh	10,264 hi	S
Liberty	6.8 fgh	343,833 fghij	11,274 hij	S
NC08-0437	4.6 def	390,333 hij	11,555 hij	S
Macana	2.2 c	344,166 fghij	11,762 hij	S
Satsumhikari	6.4 fg	333,833 fghij	12,706 hij	S
Patriot	17.6 j	274,666 efghi	14,515 ij	S
NC1880	13.4 ij	610,833 j	14,304 ij	S
NCDM04-0001	10.2 hi	653,833 ij	19,613 j	S

All table values are the mean of five replications of sweetpotato plant roots inoculated with 10,000 eggs of *M. enterolobii* and scored at 60 days after inoculation. Column values followed by the same letter are not significantly different according to Fisher's *t* test (alpha = .05) using data transformed log (x+1).

[a] Gall ratings represent visual quantification of percent of total root system with galls.

[b] Resistant (R) = sweetpotato genotypes that supported less than 500 eggs/gram root. Susceptible (S) = sweetpotato genotypes that supported more than 500 eggs/gram root

Table 4. Galling and reproduction (eggs) of *Meloidogyne enterolobii* observed in roots of selected sweetpotato genotypes in greenhouse Test 4

Genotype	Gall rating[a]	Eggs/root system	Eggs/gram root	Susceptible/Resistant[b]
L50	0 a	360 ab	4.1 a	R
Mojave	0 a	230 a	4.5 a	R
Red Resisto	0 a	398 b	7.4 ab	R
Tanzania	0 a	363 ab	9.3 b	R
Resisto	0 a	490 b	17.2 c	R
CN1058-3	3.0 bcd	35,833 c	482.3 d	R
NC17-0805	4.0 de	136,499 de	2,133 e	S
CN1058-10	4.0 de	235,000 fghi	2,848 ef	S
NC10-0275	2.0 b	231,833 d	2,869 ef	S
NC17-0806	3.2 bcd	204,833 efgh	3,169 efg	S
Tinto	2.2 bc	162,500 def	3,384 efgh	S
Ruddy	3.0 bcd	113,333 d	3,656 efgh	S
Wosaken	3.4 cd	225,666 fghi	3,642 efgh	S
Beauregard	5.6 ef	190,833 efg	4,246 fghi	S
Darby	6.4 f	281,666 ghij	4,873 fghi	S
Covington	4.0 de	168,333 def	4,478 fghi	S
Qilin	3.4 cd	201,833 efg	5,253 ghi	S
Hernandez	3.0 bcd	210,833 fghi	5,255 ghij	S
Viola	5.8 ef	305,500 fghij	7,229 hij	S
O'Henry	3.6 cd	324,666 hijk	6,014 hij	S
Kalmegh-30	6.2 ef	421,000 jk	7,922 ijk	S
Ejumula	7.8 f	615,333 k	12,264 jkl	S
L259	7.4 f	346,000 ijk	10,517 kl	S
Tinian	5.4 ef	298,666 ghijk	15,586 l	S

All table values are the mean of five replications of sweetpotato plant roots inoculated with 10,000 eggs of *M. enterolobii* and scored at 60 days after inoculation. Column values followed by the same letter are not significantly different according to Fisher's *t* test (alpha = .05) using data transformed log (x+1).

[a] Gall ratings represent visual quantification of percent of total root system with galls.

[b] Resistant (R) = sweetpotato genotypes that supported less than 500 eggs/gram root. Susceptible (S) = sweetpotato genotypes that supported more than 500 eggs/gram root.

Table 5. Galling and reproduction (eggs) of *Meloidogyne enterolobii* observed in roots of selected sweetpotato genotypes in greenhouse Test 5.

Genotype	Gall rating[a]	Eggs/root system	Eggs/gram root	Susceptible/Resistant[b]
Tanzania	0 a	64 ab	3.4 a	R
Jewel	0 a	105 bc	4.9 ab	R
TB-019	0 a	163 cd	6.5 abc	R
TB-068	0 a	205 cde	7.6 abcd	R
Centennial	0 a	56 a	7.4 bcde	R
Wagabolige	0 a	106 cd	7.4 bcde	R
Tib 11	0 a	163 de	8.6 cdef	R
TB-056	0 a	105 bcd	8.6 cdef	R
TB-146	0 a	128 cd	9.5 cdef	R
TB-257	0 a	138 cd	10.4 def	R
Dimbuka-Bukulula	0 a	230 e	10.5 ef	R
Bwanjule	0 a	168 de	11.3 ef	R
Murasaki-29	0 a	224 e	11.4 ef	R
Pelican	0 a	240 e	14.6 f	R
Processor				
Hernandez	2.6 c	81,944 f	6,429 g	S
Covington	5.0 e	213,333 g	6,506 gh	S
Ruddy	1.0 b	81,944 f	9,009 gh	S
09-0912	2.4 c	107,166 f	9,448 gh	S
TB-085	3.6 d	200,333 g	10,166 gh	S
TB-131	3.4 d	192,333 g	13,738 gh	S
Beauregard	2.8 cd	317,833 gh	12,889 h	S
Mahon-10	10.6 g	259,666 gh	23,136 i	S
TB-252	8.2 f	387,666 h	25,080 i	S

All table values are the mean of five replications of sweetpotato plant roots inoculated with 10,000 eggs of *M. enterolobii* and scored at 60 days after inoculation. Column values followed by the same letter are not significantly different according to Fisher's *t* test (alpha = .05) using data transformed log (x+1).

[a] Gall ratings represent visual quantification of percent of total root system with galls.

[b] Resistant (R) = sweetpotato genotypes that supported less than 500 eggs/gram root. Susceptible (S) = sweetpotato genotypes that supported more than 500 eggs/gram root.